SOIL BIODIVERSITY AND ECOSYSTEM FUNCTIONING

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Summary

“Essentially, all life depends upon the soil ...

There can be no life without soil and no soil without life; they have evolved together.”

– Charles E. Kellogg, 1938

Summary

Understanding the complex web of interactions in the soil communities beneath our feet and how they couple with aboveground components of ecosystems to provision ecosystem functioning proves to be one of the great frontiers of ecology. As soil-biodiversity loss is occurring on a global scale, along with increases in human populations that put a greater demand on the planet's ecosystems and biodiversity, understanding how changes in soil biodiversity will affect ecosystem functions and services is becoming a pressing issue. It is now known that soil biodiversity supports the performance of multiple ecosystem functions; and work in plant community ecology has shown how biodiversity is the key factor that can determine the stability in the maintenance of ecosystem functions over time. However, until now, there has been a gap in our knowledge on how crucial the biodiversity of soil also is in maintaining the stability of multiple ecosystem functions. This is largely because these highly complex and microscopic communities are for the most part invisible to the naked eye. Furthermore, soils still harbor many ecological unknowns due to the inherent difficulty that exists in experimentally manipulating and maintaining specific levels of soil biodiversity that can tease out the aspects of soil biodiversity that are critical for sustaining ecosystems. Despite these challenges, the goal of this doctoral dissertation is to begin to try to understand and assess the complex linkages and underlying mechanisms driving the effects of soil-biodiversity loss on the stability of multiple ecosystem functions in experimental grassland communities.

To achieve this, I first setup a simplified model grassland community in a glasshouse that tested how the presence or absence of a naturally-assembled soil community affected the asynchronous interactions, productivity and stability of a grassland plant community (Chapter 1). I was able to show that the presence of a more complex soil community increased plant

species asynchrony and stabilized plant community productivity by allowing for compensatory dynamics among plant species to occur.

In order to peer further into the black box of soil biodiversity–ecosystem functioning linkages, I experimentally manipulated soil-community diversity and composition to create a soil-biodiversity gradient that was maintained within specially-designed microcosms that minimized glasshouse-borne microbial contamination. I replicated this experimental design using soil communities from three different intensities of agricultural management practices: organic, conventional and intensive. The temporal changes in the soil microbial community as well as fluctuations in multiple ecosystem functions were regularly quantified over a 1-year period. This enabled me to demonstrate that soil-biodiversity loss has negative effects on the ecosystem functions of plant productivity, plant diversity, decomposition, soil nutrient uptake, retention and nutrient cycling, both when looked at individually, and also when combined into a single value of multifunctionality. Additionally, using the novel multiple-thresholds approach to analyze multifunctionality, I was able to highlight how the mathematical methods used to calculate multifunctionality can affect the conclusions, interpretation, and also the comparability among ecosystem functioning experiments. My results show that the soil from organically-managed field sites had higher levels of multifunctionality across the full range of ecosystem-functioning thresholds than those from conventionally- and intensively-managed sites, suggesting that organically-managed agricultural systems could be less sensitive to biodiversity loss (Chapter 2). The results from this experiment also demonstrated that soil-biodiversity loss negatively affects the temporal mean performance and stability, while increasing the temporal standard deviation of multiple ecosystem functions, including plant productivity, plant diversity, and litter decomposition. Furthermore, I was able to show that higher soil fungal taxa diversity was associated with greater negative covariance among fungal taxa over time, suggesting that richness-associated

species asynchrony in the soil could be a key factor in stabilizing ecosystem functioning (Chapter 3).

The overall results presented in my dissertation offer insight into the links that exist between soil biodiversity, patterns of soil and plant species interactions, and changes in the stability of multiple ecosystem functions. I hope that these findings might guide future experiments that eventually could lead to the development of techniques for application in real-world management efforts aiming to foster biodiversity levels that maintain the stability of the ecosystem functions on which our society depends.

Zusammenfassung

Die Wissenschaft betritt Neuland wenn es darum geht zu verstehen, wie das komplexe Interaktionsnetzwerk in den Bodengemeinschaften unter unseren Füssen funktioniert, und wie diese Gemeinschaften mit den oberirdischen Ökosystemteilen in Wechselwirkung treten, um Ökosystemfunktionen bereitzustellen. Die Bodendiversität rund um den Globus nimmt Hand in Hand mit dem Wachstum der menschlichen Bevölkerung ab. Dies bringt immer grössere Anforderungen an die Biodiversität und die Ökosysteme der Erde; daher ist gerade dieses Verständnis vom Zusammenhang zwischen Bodendiversität und Ökosystemfunktionen ein dringendes Thema. Es ist nun erwiesen, dass die Biodiversität im Boden mehrere Ökosystemfunktionen unterstützt. Ausserdem haben Arbeiten im Bereich der Pflanzenökologie gezeigt, dass die Biodiversität von Pflanzengemeinschaften einen zentralen Einfluss auf die Stabilität der Bereitstellung von Ökosytemfunktionen über die Zeit hat. Allerdings gibt es im Moment noch Lücken in unserem Wissen darüber, wie wichtig auch die Biodiversität im Boden für die Stabilität mehrerer Ökosystemfunktionen ist. Einer der Hauptgründe dafür liegt darin, dass die hochkomplexen aber mikroskopischen Bodengemeinschaften für unser Auge meist unsichtbar bleiben. Ausserdem existieren noch viele ökologisch unbekannte Eigenschaften des Bodens, weil es äusserst schwierig ist, spezifische Ebenen von Bodendiversität experimentell zu manipulieren und aufrechtzuerhalten. Dies wäre aber nötig, um Auswirkungen einzelner Komponenten der Bodendiversität zu bestimmen, welche wichtig für das Aufrechterhalten des Ökosystems sind. Trotz dieser Herausforderungen ist es das Ziel dieser Doktorarbeit, ein Verständnis dafür zu gewinnen, welche komplexen Zusammenhänge und Mechanismen dem Effekt von Bodendiversitätsverlust auf die Stabilität von mehreren Ökosystemfunktionen in experimentellen Wiesenpflanzengemeinschaften zu Grunde liegen.

Um dieses Ziel zu erreichen, habe ich zuerst ein vereinfachtes Modell einer Wiesenpflanzengemeinschaft in einem Gewächshaus erstellt und damit getestet, wie die An- oder Abwesenheit einer natürlicherweise aufgebauten Bodengemeinschaft die asynchronen Interaktionen, die Produktivität, und die Stabilität der Pflanzengemeinschaft beeinflusst (Kapitel 1). Ich konnte zeigen, dass die Anwesenheit einer komplexeren Bodengemeinschaft die Asynchronität zwischen Pflanzenarten erhöhte und gleichzeitig die Produktivität der Gemeinschaft stabilisierte, weil sie Ausgleichsdynamiken zwischen Pflanzenarten zuliess.

Um die bisher unbekannten Verbindungen zwischen Bodendiversität und Ökosystemfunktionen genauer zu durchleuchten, habe ich die Diversität und Zusammensetzung der Bodengemeinschaft experimentell manipuliert und einen Biodiversitätsgradienten geschaffen, welcher in speziell entworfenen Mikrokosmen aufrechterhalten wurde, um mikrobiellen Kontaminationen aus dem Gewächshaus vorzubeugen. Ich habe dieses experimentelle Design weiter ausgebaut, indem ich Bodengemeinschaften aus drei verschieden intensiv genutzten landwirtschaftlichen Betriebssystemen einsetzte, nämlich aus biologischem, konventionellem und intensivem Anbau. Die zeitlichen Veränderungen der mikrobiellen Bodengemeinschaft, sowie die Schwankungen von mehreren Ökosystemfunktionen, wurden regelmässig und über ein Jahr hinweg verteilt quantifiziert. Damit konnte ich zeigen, dass der Verlust von Bodendiversität einen negativen Einfluss auf die Ökosystemfunktionen Pflanzenproduktivität, Pflanzendiversität, Dekomposition, Aufnahme von Bodennährstoffen und das Rückhaltevermögen von Nährstoffen und deren Wiederverwertung hat. Diesen negativen Einfluss beobachtete ich, wenn ich die genannten Funktionen separat analysierte, aber auch, wenn ich diese in einen einzelnen Multifunktionalitätswert kombinierte. Ich konnte ausserdem durch den Gebrauch des neuen “multiple-thresholds”-Ansatzes zur Berechnung von Multifunktionalität zeigen, inwiefern die mathematischen Methoden zur Berechnung von

Multifunktionalität die Schlussfolgerungen, Interpretation und auch Vergleichbarkeit zwischen Ökosystemfunktions-Experimenten beeinflussen. Gemäss meinen Resultaten hatte der Boden von biologisch bebauten Standorten ein höheres Multifunktionalitätslevel als derjenige von konventionell oder intensiv bebauten Standorten, und zwar über die ganze Breite von Schwellenwerten („thresholds“) der Ökosytemfunktionen. Dies könnte darauf hindeuten, dass biologisch-geführte Anbausysteme weniger empfindlich gegenüber dem Verlust von Biodiversität sind (Kapitel 2). Die Resultate dieses Experimentes zeigen auch auf, dass ein Verlust von Bodendiversität sich negativ auf die durchschnittliche Leistung und Stabilität eines Ökosystems auswirkt, gleichzeitig aber die zeitliche Variabilität mehrerer Ökosystemfunktionen (inklusive Pflanzenproduktivität, Pflanzendiversität und Dekomposition von Totmaterial) erhöht. Ausserdem konnte ich zeigen, dass eine höhere Diversität von Pilztaxa mit einer grösseren negativen Kovarianz zwischen den verschiedenen Pilztaxa assoziiert war. Dies deutet darauf hin, dass Art-Asynchronität im Boden, welche mit der Diversität einhergeht, ein Schlüsselfaktor zur Stabilisierung der Ökosystemfunktion ist (Kapitel 3).

Zusammenfassend ermöglichen die Resultate dieser Doktorarbeit neue Einsichten in die Zusammenhänge zwischen Bodendiversität, Pflanzen–Bodeninteraktionen und Veränderungen in der Stabilität von mehreren Ökosystemfunktionen. So legen sie den Grundstein für zukünftige Experimente, welche zur Entwicklung nachhaltiger Anbaumethoden durch Erhaltung und Förderung von ökosystem-stabilisierenden Biodiversitätslevel führen könnten — denn schliesslich sind diese eine wichtige Lebensgrundlage unserer Gesellschaft.

General Introduction

“The nation that destroys its soil destroys itself.”

– Franklin D. Roosevelt 1937

General Introduction

Soils: full of life and mystery

Soil is a unique and fascinating field of study because of the way that it is such a familiar component of the natural world that is interwoven into our everyday lives, but at the same time is packed full of ecological mysteries that scientists have yet to truly understand. This is largely due to the fact that a soil ecosystem and most of the functions that it performs are not blatantly visible to the naked eye (Balvanera et al. 2006, van der Heijden et al. 2008). But what we cannot readily see, but are starting to understand, is how much soils represent the largest portion of the diversity of life on this planet. Soils host over one quarter of Earth's biodiversity and 98% of its genetic diversity, making the density of life and biodiversity that exists within them unmatched by other part of the natural world (Fierer et al. 2007). But the current state of the science is summarized well in a statement once made by Albert Einstein: "The more I learn, the more I realize how much I do not know". Using recent advances in rapid sequencing techniques, reports are now showing that we have only identified and can culture around one percent of soil microbial species (Bakken et al. 1997, Hibbett and Glotzer 2011), making soils as a scientific frontier matched only by that of the deep ocean ecosystem (Wall et al. 2010). This begins to highlight just how much we don't know about the specific function of soil communities and how interconnected they are with other parts of the terrestrial ecosystem (Wall et al. 2010).

Photo © Sarah Pellkofer.

A diversity of soil species found in our model systems; A) a plant root colonized by arbuscular mycorrhizal fungi, B) a nematode, and C) a collembola. Bar = 50 µm.

Soil functions and services

Beyond the value that soils hold from being the host of most of the world's biodiversity, soils are invaluable to humans because of the ecosystem functions that they drive (Wardle et al. 2004). It is now understood that soil is a crucial base for most biogeochemical cycles and natural ecosystem functions that we rely upon for ecosystem services (Wall and Lynch 2000). They are responsible for the functions that produce our food, clean our air and water, cycle nutrients and energy, regulate our climate, prevent erosion and control diseases (Bardgett and van der Putten 2014). We are just beginning to pick apart how the millions of species and billions of organisms perform different functional roles and can be divided into the guilds of species that work together to provide all of these complex services that we benefit from every day.

Degradation of soil biodiversity

But unfortunately, the stark reality of our current situation on this planet is that human populations are ever-increasing and pushing Earth closer to its carrying capacity on a daily basis. We are the most dangerous threat to the complex web of life that exists belowground. Studies have found that we have been losing the world's fertile soil at an estimated rate of 24 billion tons per year – that is 3.4 tons per person per year – mainly as a result of anthropogenic activities. This rate of soil biodiversity loss is an alarming statistic as it takes thousands of years of natural processes to make what amounts to just a couple of centimeters of soil (Soil Science Society of America 2013). And it is humanly-caused intensifications of land use, mostly in agricultural systems, which are driving a lot of this soil degradation (Matson 1997, Tilman et al. 2001). Investigations have shown that unsustainable industrial-style agricultural systems are incredibly efficient at destroying soil biodiversity through unnaturally high inputs of nutrients and fertilizers, overuse of insecticides and herbicides, as

well as physical simplification and destruction of soil-dwelling species with modern agricultural machinery and soil-manipulation practices (Brussaard 1997, Matson 1997, Tilman et al. 2001, Postma-Blaauw et al. 2010).

Soil biodiversity and ecosystem functioning

These losses in soil biodiversity, both in agricultural and natural systems, are problematic for society because we are now starting to see that soil biodiversity levels are in fact linked to the performance of multiple ecosystem functions. Recent empirical work has demonstrated that losses in soil biodiversity are powerful predictors for decreases in the performance of multiple ecosystem functions (Bardgett and van der Putten 2014, Wagg et al. 2014). And taking from the host of data on biodiversity and ecosystem functioning work that has been done involving plant communities, soil ecologists are finding that the analytical theories used to explain how biodiversity determines the performance of ecosystem functions in aboveground communities (Cardinale et al. 2012), are also applicable as the possible mechanisms driving the community dynamics underground (Brussaard 1997). Just as in plant communities, increasing biodiversity in soil communities improves the performance of many ecosystem functions as a result of species niche differentiation and facilitative interactions that allow for better use of the available biotope space and resources (Loreau and Hector 2001, Hector et al. 2002).

Quantifying ecosystem multifunctionality

More recent work has come about that provide methods for analytically calculating a single measure of the effects of biodiversity on multiple ecosystem functions simultaneously as a z-score called multifunctionality (Hector and Bagchi 2007). This measure allows for the overall performance of a system to be quantified as a single value—a practice that proves to

be helpful for making the results of complex scientific studies more understandable and accessible for non-scientific parties like politicians, the media, and the general public. However, this sort of simplification of the data is not without its flaws. Combining multiple measures of ecosystem performance unavoidably entails defining 1) if an ecosystem function is positive or negative, 2) the level of performance at which the ecosystem function is effectively functioning, and 3) the comparative value and worth of various ecosystem functions relative to each other. The inherent subjectivity of these assumptions and the possible influence from the interests of the researcher and/or stakeholders can renders the measure of multifunctionality problematic. Furthermore, many emerging studies that evaluate ecosystem multifunctionality do not abide by the same set of rules in how all of the above factors are defined and therefore this yields the results from different research groups and institutes generally incomparable, making it difficult to cross-compare work. These issues make the quest to define how soil biodiversity affects ecosystem multifunctionality a difficult one.

Soil biodiversity and the stability of ecosystem functioning

Additionally, figuring out how soil biodiversity is linked to the stability of ecosystem functioning over time is becoming more of an important issue as we are facing increased frequency and intensity of environmental change. There is a host of work showing that biodiversity in plant communities is not only positively correlated with the performance of multiple ecosystem functions, but also the temporal stability of that functioning (Isbell et al. 2009, Hector et al. 2010). I believe that the same ecological mechanisms of species interactions that drive this occurrence in aboveground communities also operate in belowground communities and can be used to better predict the effects that soil biodiversity loss will have on the stability of ecosystem functioning. I hypothesize that increasing

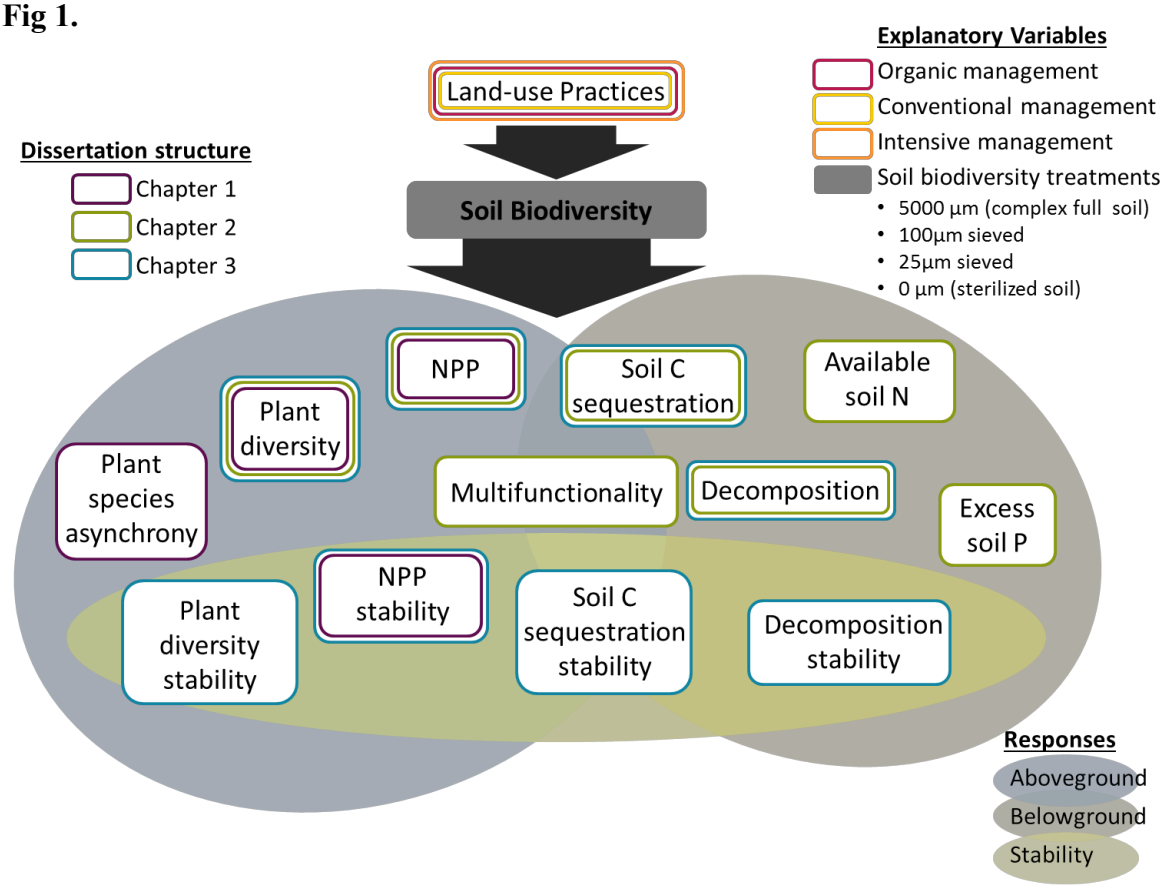
biodiversity in soil communities can mechanistically stabilize the functioning of the ecosystem by way of the so-called insurance effect (Isbell et al. 2009, Hector et al. 2010). This is due to the way that functionally diverse species respond differently to environmental changes, which results in asynchronous interactions. When this occurs in a system with high diversity, the likelihood that there will be species that perform well while when others are performing poorly is higher than when diversity is lower, leading to a greater probability for community performance to be maintained (Isbell et al. 2009, Hector et al. 2010). Empirically proving or disproving this hypothesis would hold high value for future ecological work and conservation efforts as well as for helping to maintain the stability of ecosystem services upon which we rely.

Thesis outline

To better understand the links between soil biodiversity, land-use intensity, ecosystem multifunctionality, multifunctionality stability, along with asynchronous species interactions, I setup several experiments to isolate and better associate soil biodiversity-induced differences. I first create a simplified model grassland system that I inoculate with either a natural complex soil community or a sterilized soil from one of three levels of land-use intensity (Chapter 1; Fig 1, purple boxes) and measure the effects of each treatment on the system's plant species asynchronous interactions, productivity and stability of plant productivity. Then, to better elucidate how ecosystem performance changes as a result of a spectrum of biodiversity in soil, rather than just using a presence/absence approach, I use a sieving method to experimentally manipulate soil-community diversity and composition to make a set of inocula treatments with a range of soil biodiversity. I inoculate a sterilized substrate using this range of soil-biodiversity treatments and plant a grassland plant community in a specially-designed set of fully sealed microcosms, to isolate the effects of the

inocula by preventing outside microbial influences. I monitor the temporal fluctuations in the soil microbial community along with multiple ecosystem functions over a period of one year, including plant productivity, plant diversity, decomposition, and soil nutrient cycling. To dig into the differences underlying the methodology used to quantify ecosystem functioning I analyze the performance of each function individually; and additionally I use a selection of methods to calculate a single measure of ecosystem multifunctionality (Chapter 2; Fig 1, green boxes). I perform a temporal analysis to calculate the temporal mean, the temporal standard deviation and the community stability of each ecosystem function as a response to the soil-constructed soil-biodiversity gradient treatment. I also begin to delve into the temporal changes of the soil fungal and bacterial communities as an initial effort to see how soil-species asynchrony can affect the performance and stability of multiple ecosystem functions (Chapter 3; Fig 1, blue boxes).

Fig 1.



The overall results presented in my dissertation offer insight into the links that exist between soil biodiversity, patterns of soil- and plant-species interactions, and changes in the stability of multiple ecosystem functions. I hope that these findings might guide future experiments that eventually could lead to the development of techniques for application in real-world management efforts aiming to foster biodiversity levels that maintain the stability of the ecosystem functions on which our society depends.

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Chapter 1

"We might say that the earth has the spirit of growth; that its flesh is the soil."
– Leonardo daVinci

Chapter 1

Soil Communities Promote Species Asynchrony and Stability in Experimental Grassland Communities

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Abstract

Background: Over the past two decades many studies have demonstrated that plant species diversity promotes primary productivity and stability in grassland ecosystems. Additionally, soil community characteristics have also been shown to influence the productivity and composition of plant communities, yet little is known about whether soil communities also play a role in stabilizing the productivity of an ecosystem.

Methodology/Principal Findings: Here we use microcosms to assess the effects of the presence of soil communities on plant community dynamics and stability over a one-year time span. Microcosms were filled with sterilized soil and inoculated with either unaltered field soil (complex soil community) or field soil sterilized to eliminate the naturally occurring soil biota (simplified soil community). Eliminating the naturally occurring soil biota not only resulted in lower plant productivity, and reduced plant species richness and evenness, but also destabilized the net productivity of the plant communities over time. Plant community stability was driven by changes in abundance of the dominant grass *Lolium perenne*. In contrast, the grass and legumes drove net productivity of the plant communities in microcosms where soil biota had been introduced. Additionally, the forbs showed compensatory dynamics, thus lowering temporal variation in productivity in microcosms that received the complex soil community inocula. Overall, asynchrony among plant species was higher in microcosms where a soil community had been added, which lead to higher temporal stability in community productivity.

Conclusions/Significance: Our results suggest that more complex soil communities increase plant species asynchrony and stabilize plant community productivity by equalizing the

performance among competing plant species through antagonistic and facilitative effects on individual plant species.

Keywords: Community ecology; community stability; equalizing mechanisms; plant community dynamics; plant productivity; plant–soil (below-ground) interactions; plant species asynchrony

Introduction

Understanding the mechanisms behind biodiversity–ecosystem functioning relationships is a major issue in ecology for predicting and maintaining ecosystems in the face of environmental change (Díaz et al. 2006, Rockström et al. 2009, Cardinale et al. 2012, Hooper et al. 2012). Previously it has been shown that higher levels of species diversity, specifically in grassland ecosystems, can maintain ecosystem functioning, and in particular primary productivity (Hooper 1997, Hector et al. 1999, Loreau et al. 2001, Cardinale et al. 2002, Hector and Bagchi 2007). Several studies also showed that performance and stability of net primary productivity (NPP) of an ecosystem are directly linked to plant community diversity and composition (McNaughton 1977, Naeem et al. 1994, Tilman et al. 1996, 2006, Isbell et al. 2009, Hector et al. 2010, Roscher et al. 2011b). In general, greater stability in ecosystem NPP at higher levels of plant species richness can be linked to the increased likelihood for species to respond asynchronously to environmental perturbations, thus stabilizing the overall performance of the community through time (Loreau 2010, de Mazancourt et al. 2013). This can be associated with the increased probability of niche differentiation that occurs among the species at higher richness levels (Huston 1979, Chesson 2003, Loreau and de Mazancourt 2008, Hector et al. 2010).

Considering the importance of plant species diversity in stabilizing NPP throughout environmental perturbations, it is critical to consider ecological mechanisms that support plant community diversity and mediate their temporal performance. For instance, soil communities are well known to influence multiple ecosystem functions (van der Heijden et al. 1998, 2008, Wardle et al. 2004, Wagg et al. 2014), with particular effects on plant competition and the overall performance and composition of a plant community (van der Heijden et al. 1998, 2008, Wardle et al. 2004, Wagg et al. 2011a, 2014, Hendriks et al. 2013).

Considering that diversity and composition of the soil community have a strong influence on the performance of individual plant species and plant community composition, it is likely that soil communities may be an underlying mechanism influencing the stability of plant community productivity. Thus soil organisms that alter the performance of individual plant species within a community could potentially increase or decrease the stability of plant community productivity through altering temporal competition dynamics among the plant species as the plant community develops and responds to environmental variation (Van der Putten and Peters 1997). This is of critical importance since it is now known that many anthropogenically managed ecosystems show altered soil community composition as well as the suppression and loss of key groups of soil organisms that can alter the plant community performance and composition (Mäder et al. 2002, Postma-Blaauw et al. 2010, Verbruggen et al. 2010, Moora et al. 2014, De Vries et al. 2015). Only recently, there has been some evidence to suggest that the suppression of key soil biota, such as mycorrhizal fungi, may be linked with stability in NPP (Yang et al. 2014). However, there is currently little evidence to know whether soil communities overall influence plant community stability.

Here we investigate the importance of the soil community for supporting temporal stability in the NPP of a grassland plant community and the temporal asynchrony among plant species as the plant community develops. Considering the connections previously found between the presence of soil biota and plant community performance, we hypothesize that a presumably complex soil community which naturally co-occurs with a plant community will not only support a high diversity and NPP in the plant community, but will also promote its stability in productivity via increased plant species asynchrony. To address our hypothesis, we established a grassland plant community in a standardized sterile soil substrate inoculated with either a natural unaltered soil community (“complex soil community”), or the same inoculum, but sterilized to remove the natural soil biota. For simplicity, we refer to the sterile

treatment a “simplified soil community”. Plant productivity, community composition, species asynchrony, and community stability were assessed every two and a half months in the experimental communities over a one-year period to determine the role of soil biota in stabilizing the performance of the plant community.

Materials & Methods

Soils and inocula

Experimental microcosms were set up using 42 three-liter pots (19 cm diameter x 14.5 cm height) that were sterilized by autoclaving. Each pot was filled with 2.25 kg (dry mass) substrate of a 50/50 field soil/quartz sand mix that was sieved through a 5 mm mesh and sterilized by autoclaving (120 °C for 90 minutes). The field soil used as the sterile substrate in each microcosm came from a natural grassland near the Agroscope Reckenholz research station in Zürich, Switzerland (47° 25’ 38.71” N, 8° 31’ 3.91” E). The sterilized field soil was inoculated with 125 g of one of the six possible inocula treatments: soil inoculum from three sites with different management practices × two soil community treatments — unaltered (complex) or sterilized (simplified). The inocula were mixed throughout the substrate prior to planting. Each of the six soil inocula treatments was replicated seven times for a total of 42 experimental communities.

The soil inocula were collected from three agricultural fields with different management histories. We used soils from these different management practices to better generalize our results independent of site-specific histories and characteristics. All sites from where our study's soil samples were collected did not host endangered or protected species. With the permission of Jochen Mayer of Agroscope and Paul Mäder of the Institute of Organic Agriculture (FiBL), we were allowed to collect two of the soils from FiBL’s so-called DOK experimental field site in Therwil, Switzerland (47° 30' 8.9964” N, 7° 32' 21.8292” E). This experiment was designed to assess different agricultural management practices, such as conventional and organic management, on various ecological and agricultural characteristics of plots (see [29] for details). For the present study soil was collected from four plots where the management practice was the addition of organic

fertilizer (Site A, organic) and from another four plots where the management practice was addition of mineral fertilizer (Site B, conventional). The third soil was sampled, with the permission of the land owner, Georg Schitterer, from his privately-owned agricultural plot in Freiburg, Germany (47° 58' 26.058" N, 7° 46' 31.5336" E). This site had been continuously planted with the same crop species (maize) for more than 10 years (Site C, intensive). Details about soil characteristics of the different soil treatments are provided in S1 Appendix in Supporting Information.

At all three sites soil was collected using four transects, one meter apart per plot, coring soil every four meters. Soil cores were mixed per site and homogenized by sieving through a 5 mm sieve. Half of the soil from the three sites was sterilized by autoclaving (120 °C for 20 min). This resulted in two inocula treatments per site; a sterile inoculum with a "simplified" soil community and an un-sterile inoculum which we refer to as the more "complex" soil community (*sensu* 27,28,35). Autoclaving soil is well known to eliminate the presence of mycorrhizal fungi and severely reduce the microbial community (Tiwari et al. 1988, Carter et al. 2007, Lau and Lennon 2011, Wagg et al. 2014). The inocula volume only made up approximately 5% of the total soil volume to minimize the possible abiotic effects of inocula sterilization in our model systems. We used root colonization by arbuscular mycorrhizal fungi (AMF) as a proxy for the condition of the soil communities under the two soil treatments to confirm the soil communities of the autoclaved treatment remained after 1 year. Although AMF colonization is only one component of soil community composition, the absence or presence of AMF can act as an effective indicator as to the state of the community composition of the soil community in that a key component of the soil microbiota have been effectively eliminated or severely suppressed. AMF colonization was highly different between the complex and simplified soil inocula treatments ($F_{1, 37} = 122$, $P < 0.0001$). AMF colonization in the sterile treatment was on average 5.67 % and was not statistically different

from zero (95 % confidence interval = - 0.23 to 11.1). Conversely the complex soil community treatment had a mean colonization of 60.4% (95% confidence interval = 54.9, 65.8). This suggests that the differences in the soil communities resulting from our two soil inocula treatments were largely maintained.

Plant community

In Fall of 2012 each microcosm was planted with six individuals of the grass *Lolium perenne* and six individuals of the nitrogen-fixing legume *Trifolium pratense*, along with one individual of *Achillea millefolium* (forb), *Festuca pratensis* (grass), *Lotus corniculatus* (legume), *Plantago lanceolata* (forb), and *Prunella vulgaris* (forb), for a total of 17 individual plants per pot. These plant species commonly co-occur in European grasslands (Lauber et al. 2012). Moreover, this specific mixture made up largely of *T. pratense* and *L. perenne,* was selected because the two main species commonly co-occur and are extensively used in land management as crop in fallow years on agricultural fields or establishment as fodder crops. Additionally, *T. pratense* and *L. perenne* are model species for studying temporal dynamics in plant communities due to their complementary use of the biotope that results in their overyielding (Nyfeler et al. 2008, Lüscher et al. 2008). Moreover, legumes depend heavily on associations with their soil biota for increased performance (Klironomos 2003, Wagg et al. 2011a, 2014). We included the five other plant species in the experimental communities at a lesser abundance because they commonly occur in managed grass-clover fields, and they also allow for a better assessment of plant community compositional responses (e.g. richness, evenness).

Seeds of each species were surface sterilized by immersion in 2.5 % hyposodium chlorate for five minutes, then rinsing thoroughly in distilled H_2O. Surface-sterilized seeds were then plated onto 1% Agar in Petri dishes to germinate. In order to ensure that the seeds

of all species were at the same stage of development when planted, the seed germination process was staggered so that each species exhibited the presence of cotyledon(s) and/or radicle when transplanted. Seedlings were planted into one of 17 evenly spaced and randomly selected positions in the inoculated substrate of each microcosm. These experimental communities were set up over two days and the day on which each was set up was used as a blocking factor in the subsequent analysis of variance (ANOVA).

These experimental communities were established in a greenhouse compartment where natural light was subsidized by 400-W high-pressure sodium lamps in order to maintain an environment of 16 h / 25 °C days and 8 h / 16 °C nights with a light level above 300 W/m2. Twice weekly, the microcosms were watered to maintain gravimetric soil moisture in the range of 10–20 %. However, since the greenhouse conditions maintain a constant environment, which does not reflect those found in nature which might allow for variation in plant species competitive interactions through time, we induced a variation in the watering regime to simulate an extended period without rain. The variation in precipitation was applied to all of the experimental communities at the same time by withholding watering for 10 days beginning five and a half weeks before each harvest. The plant communities were grown under these conditions for a total of 55 weeks (~1 year), with five harvests starting 11 weeks after planting and occurring every 11 weeks after that.

Data collection

Over the 55-week growing period plant individuals were cut at 5 cm above the soil surface every 11 weeks to simulate hay making, the regular procedure of harvesting in these grasslands. Plants were harvested from the experimental communities according to the same schedule in which they were planted. Plant individuals were counted and separated by species, dried at 65 °C and the biomass weighed. For each harvest we calculated the total

biomass (net primary productivity = NPP), plant realized species richness and Pielou's evenness index ((Pielou 1975), $J' = \frac{H'}{H'_{max}}$, where *H'* is derived from the Shannon diversity index (the sum of the proportion of a species times the log proportion) and H'_{max} is equal to $H'_{max} = -\sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S$ per *i* species). Plant species asynchrony was derived for each experimental community as $1 - \varphi_b$, where φ_b is species synchrony, calculated by $\varphi_b = \frac{\sigma^2}{(\sum_{i-1}^{S} \sigma_i)^2}$, where σ^2 is the variance in NPP over time and σ_i is the temporal standard deviation of the *i*-th species in each experimental community as defined by Loreau & de Mazancourt (Loreau and de Mazancourt 2008). Since our experimental design utilized a plant community dominated by a common grass-clover mixture, we also assessed the asynchrony among plant functional groups using the above-mentioned equation for asynchrony with σ^2 being the variance in the sum of the biomass of two compared plant functional groups and σ_i as the temporal standard deviation of plant functional group *i*. Considering this additional level of community grouping, beyond the individual species, has been shown to be of particular importance for capturing a more accurate picture of the effects of diversity on ecosystem stability (Flynn et al. 2011). We calculated temporal stability in both NPP of the whole community and of each individual plant species using the inverse coefficient of variation determined by μ/σ, where μ is the overall temporal mean of each community's or species' NPP and σ is the standard deviation of NPP over time (Loreau and de Mazancourt 2008, 2013, de Mazancourt et al. 2013). To understand the effect of species *i* on the temporal variation in the NPP of a microcosm over time we assessed the covariance of a species biomass (N_i) with the NPP of the community over time; $cov(N_i, NPP)$, where the sum of the species covariance with NPP through time is the temporal variance in NPP since $\sigma^2_{NPP} = (NPP - \overline{NPP})^2 = \sum cov(N_i, NPP)$.

Data analysis

All data analysis and statistics were completed using R software (version 3.0.0) and the packages 'vegan', 'lme4', and 'lmerTest' (R Development Core Team 2011). In all analysis significance was determined as a type I error of $\alpha < 5$ %. Linear mixed-effects models were used to assess the variation in plant community characteristics with the experimental block and the field site from where soil inoculum was collected as random effects in all ANOVA models. The field site from where each soil sample was collected was also considered and assessed as a fixed effect in the models.

Plant community characteristics that were repeatedly measured throughout the experiment (NPP, evenness, richness) were assessed for variation between soil community treatments (complex and simplified) and the interaction with the harvest time point using mixed-effects models as mentioned above but with the identity of the microcosm added as a random effect to account for repeated measures. However, since we were specifically interested in the general effects of the soil community on the temporal performance of the plants in a community context, the management history was ultimately set as a random effect and its interaction with the soil sterilization treatment. (but see S2–S5 Appendix for site-specific effects). Additionally, we included the density of individual plants within the ANOVA model ahead of all fixed effect terms to counteract density-dependent performance of plants that may have influenced these plant community characteristics. NPP was square-root transformed prior to analysis to meet model assumptions. The temporal stability in the NPP and in the performance of individual plant species, the contribution of individual species to temporal variation in NPP, as well as the temporal asynchrony among plant functional groups were assed for differences between complex and simplified soil community treatments with only the soil community treatment as a fixed effect in the model. Additionally, we included an assessment of the temporal standard deviation of NPP under

each soil community treatment, as previous work has shown that it is a useful tool for understanding if differences in the outcomes of the stability calculation (μ/σ) are driven more by differences in the mean or the temporal variation of each treatment (Gross et al. 2014, Hautier et al. 2015).

Results

The simplified soil community resulted in lower net productivity of the plant communities (Table 1, Fig 1a & Fig 2a), which was particularly stronger at some harvest points than others as indicated by the soil inocula treatment by harvest interaction (Table 1; Fig 1a). The overall performance of the communities and their variation overtime is a consequence of the response of the individual plant species to the soil inocula treatments, where generally, all the plants species were less productive with the simplified soil community with the exception of the predominant grass *L. perenne* (Fig 1b–h & Fig 3, S7 Appendix). Plant species richness and evenness were also reduced by the simplification of the soil communities as a consequence of the reduction in the productivity in the majority of the plant species at a benefit to the productivity of the grass *L. perenne* (Table 1, Fig 2b–c). Moreover, NPP and plant species evenness and richness also varied through time (Table 1), indicating temporal dynamics in the plant community characteristics. More specifically, the complex soil community resulted in greater temporal stability in NPP ($F_{1, 37} = 14.4$, $P = 0.0005$, Fig 2d), greater asynchrony among individual plant species through time ($F_{1, 37} = 9.19$, $P = 0.004$, Fig 2e), as well as lower temporal standard deviation in NPP ($F_{1, 37} = 4.65$, $P < 0.05$; Fig 2f).

Table 1. ANOVA results for the effects of soil treatment (simplified versus complex) and harvest time on NPP, evenness and realized richness of 42 microcosms (random term).

		NPP		Evenness		Richness	
	DF_{num}	DF_{den}	F	DF_{den}	F	DF_{den}	F
Density[†]	1	159.1	29.0***	175.0	22.3***	181.8	131.1***
Harvest (H)	4	163.1	37.7***	162.8	66.5***	162.6	3.90 **
Soil (S)	1	2.63	11.1 **	2.57	102.1***	2.52	32.4***
H × S	4	162.3	4.80**	162.0	2.27	161.9	2.66*

[†]The number of individual plants per microcosm at each harvest ncluded to account for density dependence of response variables'* = P < 0.05, ** = P < 0.01, *** = P < 0.001;DF_{num} = numerator degrees of freedom, DF_{den} Kenward-Roger adjusted denominator degrees of freedom, F = F-ratio.

Fig 1. Mean biomass and standard errors of the mean are shown for (a) NPP and (b–h) the individual plant species at each harvest when grown with a simplified (light points, dashed line) or complex (dark points, solid line) soil community.
Lines connecting means highlight the trend between consecutive harvest time points.

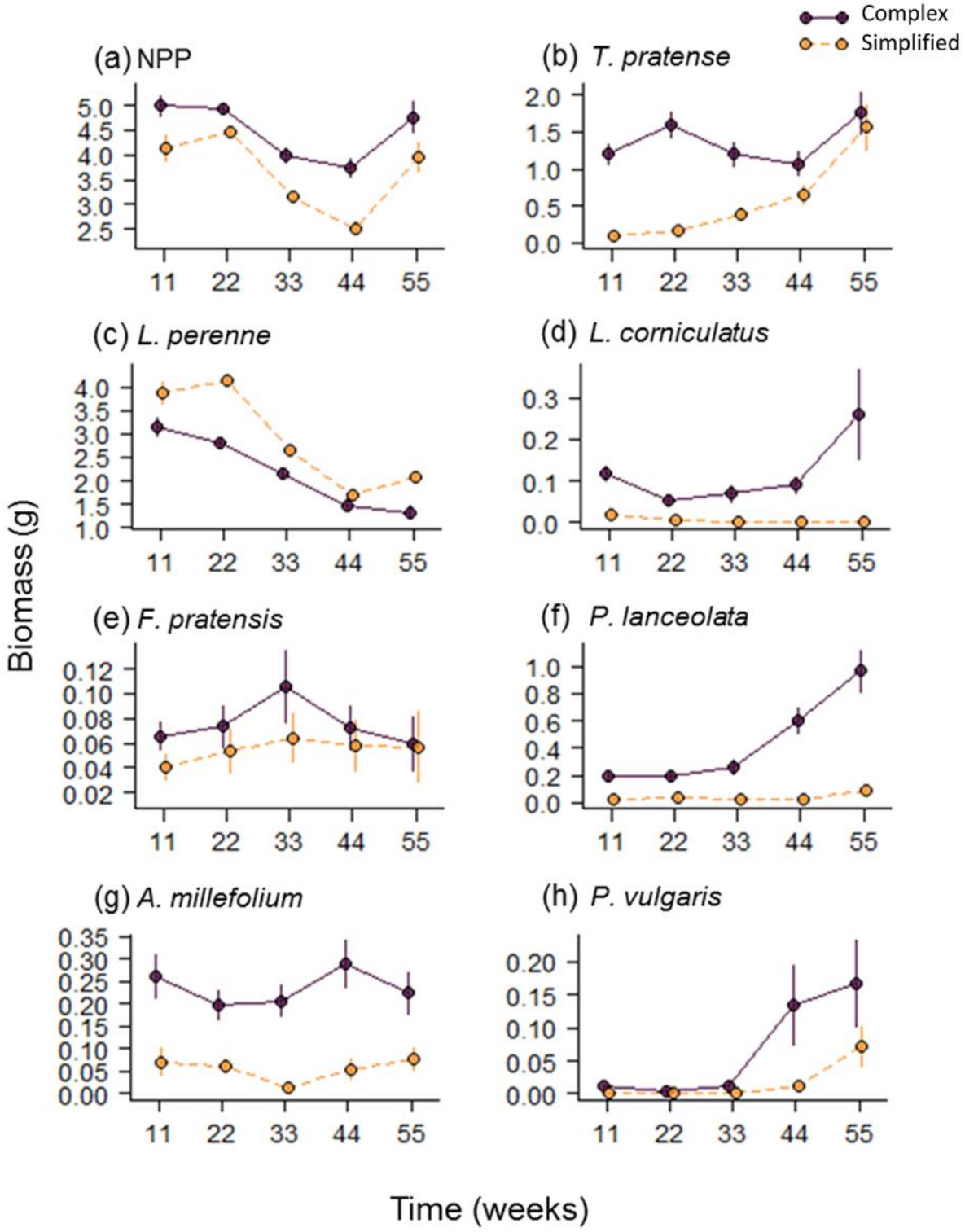


Fig 2. Means with 95% confidence intervals for the pair-wise difference between the complex and simplified soil community treatments are shown for (a) NPP, (b) realized plant species richness, (c) plant species evenness, (d) temporal stability in NPP, (e) asynchrony among plant species through time, and (f) the temporal standard deviation of NPP under the complex (dark points) and simplified (light points) soil community treatments.

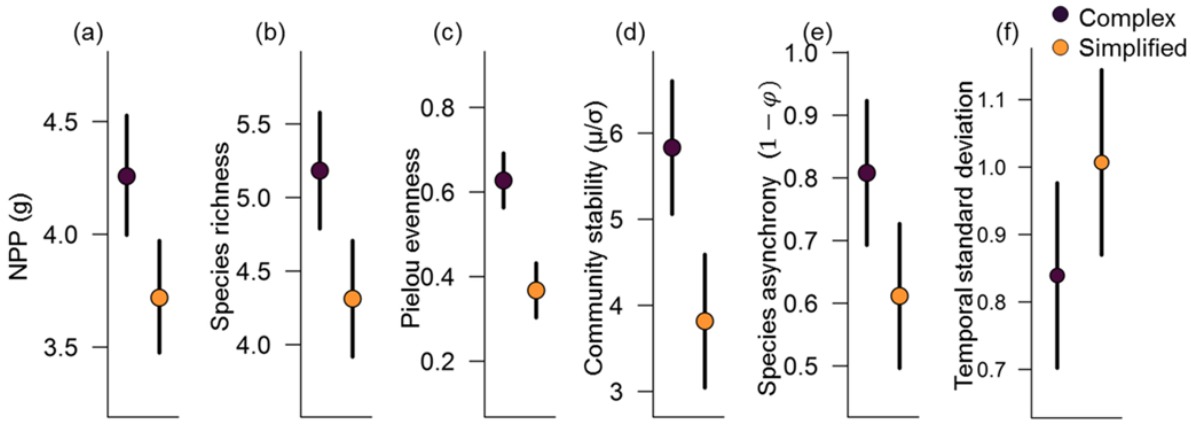
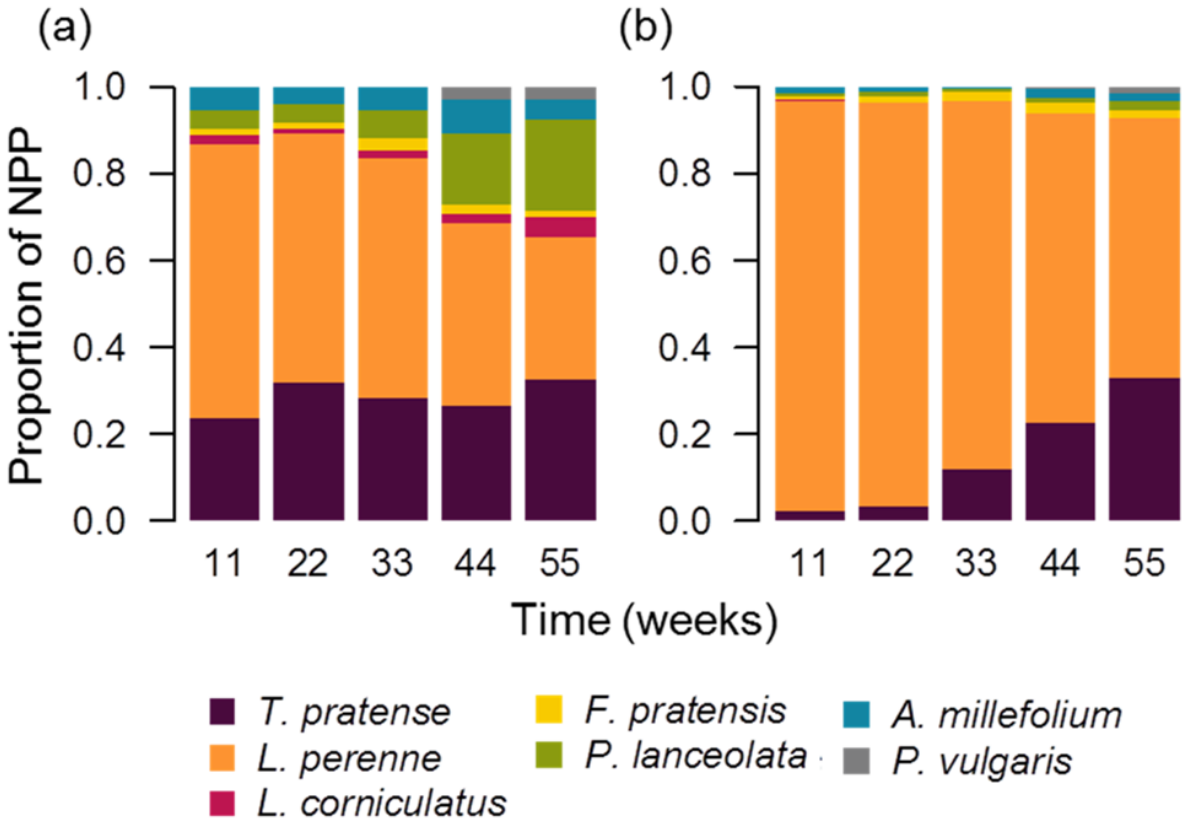


Fig 3. Plant species proportions of community NPP for each harvest time with (a) the complex soil community and (b) the simplified soil community.
Colored bar height indicates the proportion of community NPP made up by each plant species.



Although species asynchrony was greater with the more complex soil, individual plant species were also generally less variable over time with the complex soil community (Table 2, Fig 4a). Specifically, the stability in the performance of *A. millefolium* and the legumes *L. corniculatus* and *T. pratense* were most negatively affected by the simplification of the soil communities (Fig 4a). Moreover, the complex soil community resulted in a lower contribution of *L. perenne* to the overall variation in NPP, while the contribution of *T. pratense* was marginally increased (Table 2; Fig 4b). Therefore, the lower stability and species asynchrony in the simplified soil community was largely driven by the temporal variation in the performance of *L. perenne* (Fig 4b). This indicates that in the simplified soil community the variation in the performance of *L. perenne* was not sufficiently compensated by the variation in performance of the other species, thus constant NPP could not be maintained across harvests. This is evidenced by the dominance of the grass within these communities (e.g. Fig 3b), such that temporal trends in the variation in NPP and performance of *L. perenne* across harvests were highly similar (see Fig 1a and c). Conversely, with the complex soil community, the variation in individual plant species performances was better able to compensate for temporal fluctuations of the dominant grass *L. perenne* as indicated by the greater species asynchrony (Fig 2e).

Table 2. ANOVA results for the effect of the soil community treatments on the stability in the biomass of individual species and the covariance between the individual plant species and NPP.

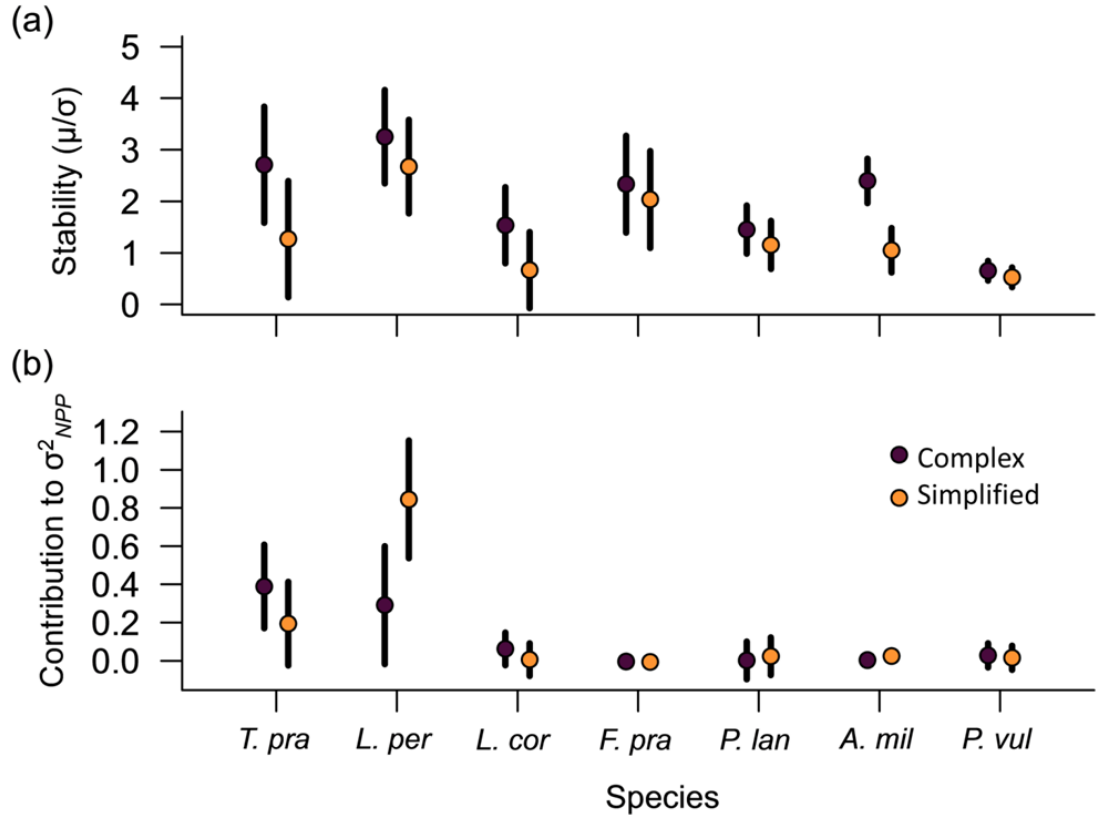
			Stability		*cov(N_i, NPP)*
	DF_{num}	DF_{den}	F	DF_{den}	F
T. pratense	1	36.0	6.81 *	37	3.24 †
L. perenne	1	37.0	1.67	37	13.4 ***
L. corniculatus	1	29.1	5.77 *	37	1.79
F. pratensis	1	29.7	0.42	37	0.05
P. lanceolata	1	32.2	1.65	37	0.19
A. millefolium	1	29.0	41 .0 ***	37	2.23
P. vulgaris	1	16.8	1.99	37	0.15

† = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

DF_{num} = numerator degrees of freedom, DF_{den} Kenward-Roger adjusted denominator degrees of freedom, F = F-ratio.

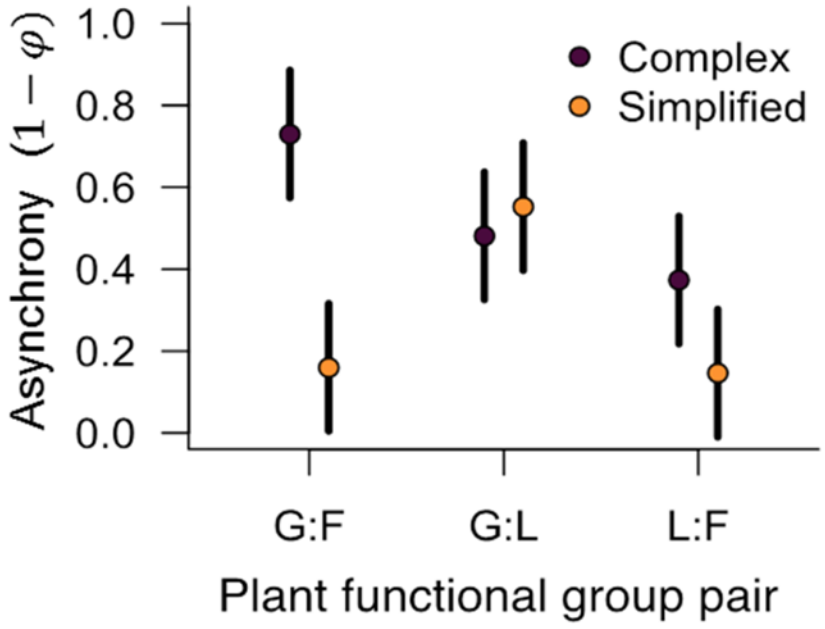
Note, only the effect of soil treatment is shown for simplicity, but see S5 Appendix for full ANOVA results.

Fig 4. Mean values with 95% confidence intervals for (a) the stability in performance of individual plant species and (b) the contribution of each to the temporal variation in community NPP, calculated as the covariance between NPP and the biomass of a species with the complex (dark points) and simplified (light points) soil community treatments. Plant species names are abbreviated on the x-axis.



The effect of the soil community treatment on the asynchrony between different functional groups depended on the functional group pairing (Fig 5, $F_{2,80}$=16.2, P<0.0001). Specifically, the more complex soil community promoted asynchrony between grasses and forbs and between forbs and legumes but not between grasses and legumes (Fig 5).

Fig 5. Mean asynchrony between pairs of plant functional groups with 95 % confidence intervals for the complex (dark points) and the simplified (light points) soil community treatments.
(G = grasses, F = forbs, L = legumes)



Discussion

It has been well documented that plant community composition is altered by various soil biota, such as pathogens, decomposers and symbiotic fungi (Francis and Read n.d., Van der Putten and Peters 1997, van der Heijden et al. 1998, Hendriks et al. 2013, Eisenhauer et al. 2013, Wagg et al. 2014, De Vries et al. 2015). Paralleling these past studies, we found that the more complex soil community maintained higher plant species richness and resulted in a more even plant community with greater NPP than plant communities with a simplified soil community. More importantly, in line with our hypotheses, we found that the more complex

soil communities led to higher stability of community NPP and maintained a higher asynchrony among plant species than the simplified soil community as our experimental plant communities developed over the course of the experiment. Our analysis of the temporal standard deviation pointed out that the higher stability we found in the NPP with the more complex soil community resulted from a combination of both a higher temporal mean NPP and a lower temporal standard deviation in these systems. However, the greater differences in the temporal mean between the treatments had a slightly stronger influence in the stability calculation.

Greater stability in more species-rich grassland communities is often observed to be associated with lower stability in the performance of individual plant species due to strong asynchronous fluctuations among plant species that result from combinations of environmental, demographic and competitive fluctuations (Tilman et al. 1998, Chesson 2000, Loreau and de Mazancourt 2008, de Mazancourt et al. 2013). However, unlike previous studies, we found greater stability in the performance of individual plant species in communities where the overall NPP was more stable. Moreover, with the simplified soil community the temporal variation of NPP of the plant community was largely driven by the dominance of the grass *L. perenne* such that the temporal variation in the subdominant species had little effect in stabilizing NPP across time. Therefore, the fluctuations in species biomasses could not compensate for the proportionally larger fluctuations in performance of *L. perenne* at different time points. This corresponds with previous findings that a higher evenness in the performance of plant species, often as a result of greater plant species richness, is a key component behind the stability in the NPP of a community and may be suggestive of greater species asynchrony (Doak et al. 1998, Cottingham et al. 2001, Roscher et al. 2011a, Thibaut and Connolly 2013).

The asynchrony among plant species as well as between plant functional groups in our study likely reflect variations in the temporal competitive interactions that are driven, either directly or indirectly, by facilitative and antagonistic effects of the soil community on the performance of individual plant species as our plant communities developed overtime. The influence that soil biota can have on the performance of individual plant species has been well known to shift plant–plant competitive interactions and ultimately community composition(Hartnett et al. 1993, Hetrick et al. 1994, Zobel and Moora 1995, Van der Putten and Peters 1997, Scheublin et al. 2007, Wagg et al. 2011c, Hendriks et al. 2013, De Vries et al. 2015). Therefore, the greater richness and evenness in our plant communities could have been a direct effect of soil biota, such as via mycorrhizal fungi and rhizobia required for improved growth of legumes, as well as plant species-specific soil pathogens that could have reduced the performance of the grass *L. perenne*. Thus, at the same time soil organisms were likely indirectly affecting competitive interactions among plants by benefiting or inhibiting the resource acquisition of species as the plant communities utilized more of the biotope throughout the development of the communities. In support of this concept, van der Putten & Peters (Van der Putten and Peters 1997) observed that competition between two grasses over a 16-week period was altered by the sterilization of rhizosphere soil biota. They found that the competitive suppression of the subdominant plant over time was increased by sterilization of rhizosphere biota similar to more recent findings of Hendricks *et al.* (Hendriks et al. 2013). Recently, Yang *et al.* (Yang et al. 2014) also reported that suppression of mycorrhizal fungi altered the dominance of particular plant species, and reduced the performance of N-fixing forbs and the overall temporal stability in the performance of a grassland ecosystem. Considering these studies, it would seem that the temporal variations in the performance of individual plants species can be driven by the soil community, both directly and indirectly, through beneficial and antagonistic plant association that potentially drive differences in the

competitive interactions among plant species and therefore their asynchrony and the overall net community stability.

Overall our results indicate that the complexity of the belowground soil community with which plants interact can influence the temporal performance of individual plant species and potentially the competitive interactions among plants. This leads to greater species asynchrony and the overall stability in the performance of the plant community (Tsiafouli et al. 2015). Furthering such findings in the future may be of key importance for land management practices where the diversity and the presence of various groups of soil biota are frequently found to be suppressed by increased anthropogenic activity (Mäder et al. 2002, Postma-Blaauw et al. 2010, Verbruggen et al. 2010, Moora et al. 2014). However, additional efforts are needed to better elucidate the more finite mechanisms by which the various components of the soil community (i.e. pathogens or mutualisms) drive asynchrony among plant species and stabilize ecosystem NPP in both managed and unmanaged ecosystems.

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Supporting Information

Additional supporting information may be found in the online version of this article:

S1 Appendix. Inocula soil history with initial soil properties analyses results.

Term	Management	Site name & location	pH	NH_4^+ *	NO_3^- *	P-Test *	K-Test *	Mg-Test *	P *	K *	Mg *	Ca *
Site A, organic	organic fertilizer, "bio-Organic"	DOK† trial, Therwil, Switzerland	7.9	41.3	<0.5	4.3	1.9	9.9	50.3	61.1	206	6228
Site B, conventional	mineral fertilizer, "Konventionell"	DOK trial, Therwil, Switzerland	7.4	42.6	1.8	3.2	2.0	10.4	47.5	59.7	202	6074
Site C, intensive	Intensive, 10-year maize mono cropping	Private farm, Freiburg, Germany	7.4	44.3	<0.5	4.3	1.7	10.8	41.1	65.0	216	6533

*Results in mg/kg. Water soluble inorganic N (NH_4^+ and NO_3^-) in dry soil was determined by a Skalar segment flow analyzer (Skalar, Breda, NL), plant available phosphorus (P-Test), potassium (K-Test) and magnesium (Mg-Test) content were extracted with ammonium acid-extraction, CO_2 and $CaCl_2$, and mg/kg P, K, Mg, and Ca amounts were determined using ammonium acetate-EDTA (pH 4.65) extraction.

† Biologisch-dynamisch, organisch-biologisch and konventionell (DOK) trial. See www.fibl.org for more information about the study design (Mäder *et al.* 2002).

S2 Appendix. Results of mixed-effects analysis of variance (ANOVA) for resulting NPP, evenness and richness, considering density (the number of individual plants in each community), harvest, the soil inocula treatment, the site source of the soil and all combinations of the interaction between harvest, soil treatments and site as fixed effects and the 42 microcosms as random-effects term.

ANOVA			**NPP**[†]	**Evenness**	**Richness**
	df	denDF	F	F	F
Density	1	143	74.8***	25.8***	264***
Harvest (H)	4	143	40.7***	78.7***	3.70 **
Soil (S)	1	35	15.6 ***	92.8 ***	37.9 ***
Site (Si)	2	35	0.072	5.82**	7.29**
H x S	4	143	5.15	2.25	2.73*
H x Si	8	143	0.682	0.430	1.65
S x Si	2	35	1.96	0.055	0.977
H x Si x S	8	143	2.89**	1.16	0.727

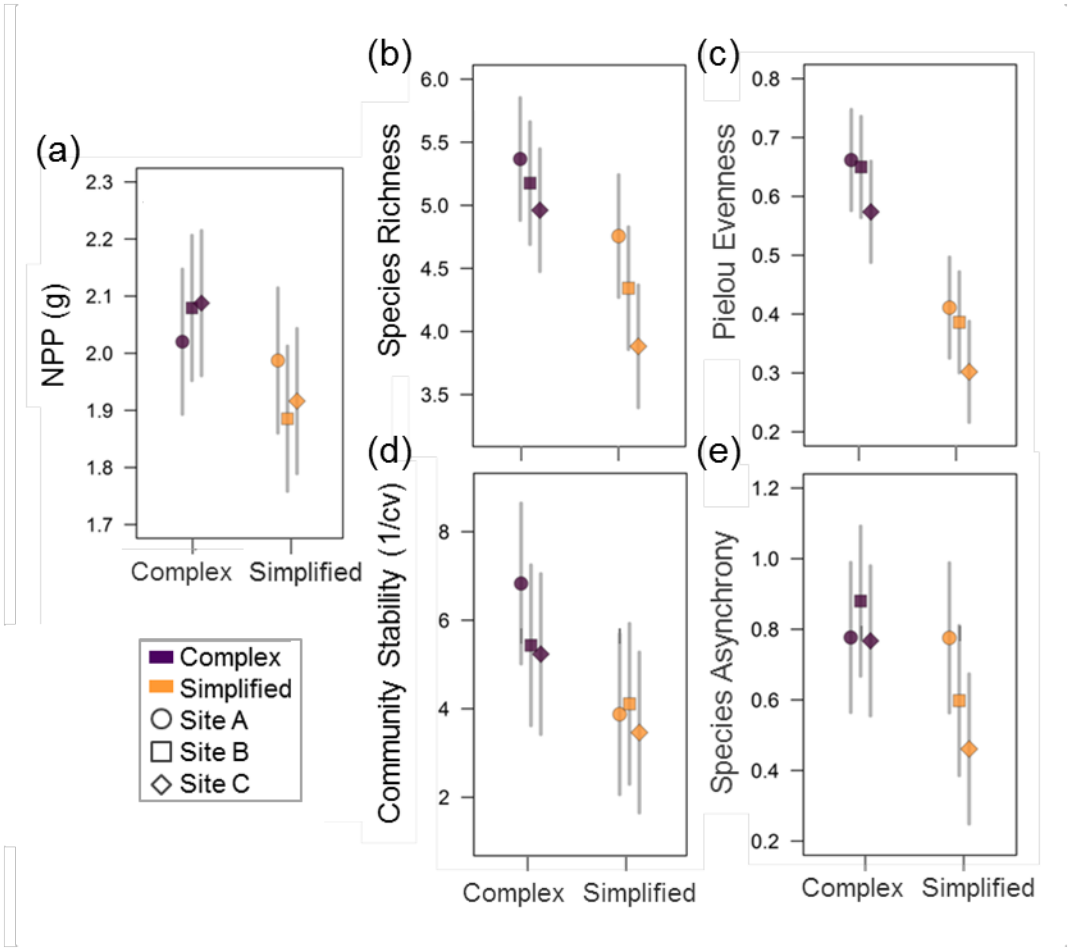
[†] Square root transformed.
* = P < 0.05, ** = P < 0.01, *** = P < 0.001
df = degrees of freedom, DF_{den} = Kenward-Roger adjusted denominator degrees of freedom (of error term), F = F-variance ratio.

S3 Appendix. Results of mixed-effects analysis of variance (ANOVA) for resulting community stability and species asynchrony (both untransformed) considering the soil inocula treatments and the site separately.

ANOVA			**Community Stability**	**Species Asynchrony**
	df	denDF	F	F
Soil (S)	1	35	14.3***	9.90**
Site (Si)	2	35	1.19	2.47
S x Si	2	35	0.83	2.46†

† = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001
df = numerator degrees of freedom, DF_{den} Kenward-Roger adjusted denominator degrees of freedom (of error term), F = F-variance ratio.

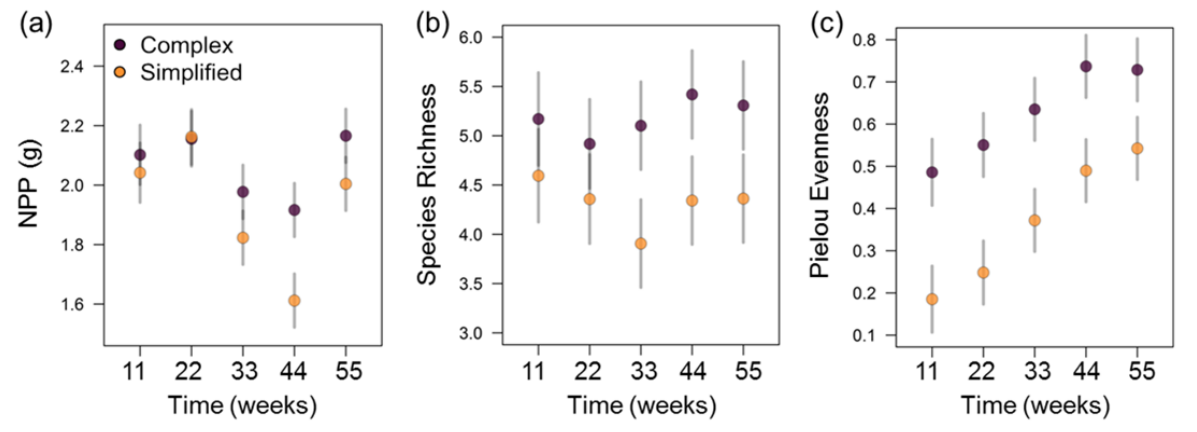
S4 Appendix. Mean values with 95% confidence intervals of the (a) NPP, (b) richness, (c) evenness, (d) community stability, and (e) species asynchrony of plant communities with a complex soil community (dark points) and simplified soil community (light points) for the three sites over the full duration of the experiment.



S5 Appendix. Full ANOVA results for the effect of the soil community treatments on the stability in the biomass of individual species and the covariance between the individual plant species and NPP, including the interaction of soil treatment and site origin.

				Stability		$cov(N_i, NPP)$	
Species	Treatment	numDF	denDF	F	P	F	P
T. pratense	Soil (S)	2	35	7.21	0.011	0.46	0.635
	Site (Si)	1	35	0.73	0.491	3.23	0.081
	S x Si	2	35	2.10	0.138	1.47	0.244
L. perenne	S	2	35	1.91	0.175	2.23	0.123
	Si	1	35	2.61	0.088	12.58	0.001
	S x Si	2	35	3.75	0.033	0.10	0.905
L. corniculatus	S	2	35	5.32	0.029	1.86	0.171
	Si	1	35	0.26	0.774	1.89	0.178
	S x Si	2	35	0.67	0.521	2.05	0.144
F. pratensis	S	2	35	0.37	0.549	0.06	0.942
	Si	1	35	0.98	0.389	0.05	0.826
	S x Si	2	35	0.62	0.546	0.63	0.538
P. lanceolata	S	2	35	1.55	0.223	0.18	0.834
	Si	1	35	3.80	0.034	0.18	0.671
	S x Si	2	35	3.13	0.058	0.66	0.525
A. millefolium	S	2	35	38.4	1.8e-6	0.21	0.814
	Si	1	35	0.11	0.900	2.09	0.157
	S x Si	2	35	0.02	0.977	0.53	0.593
P. vulgaris	S	2	35	0.54	0.595	1.03	0.366
	Si	1	35	2.39	0.145	0.15	0.700
	S x Si	2	35	0.88	0.365	1.14	0.330

S6 Appendix. Mean values with 95% confidence intervals of plant (a) NPP, (b) richness, and, (c) evenness for each harvest the complex (dark points) and simplified (light points) soil community treatments.



S7 Appendix. ANOVA results for the variation in the biomass of individual plant species among harvest time points (Harvest) and soil community (Soil) treatments.

	numDF	denDF	MS	F value
T. pratense				
Density‡	1	164.17	19.05	63.62 ***
Harvest (H)	4	163.05	9.28	31.00 ***
Soil (S)	1	43.81	2.51	8.40 **
H x S	4	162.25	1.06	3.55 **
L. perenne				
Density	1	163.88	3.37	11.96 ***
Harvest (H)	4	163.03	35.47	125.93 ***
Soil (S)	1	43.82	4.78	16.96 ***
H x S	4	162.23	1.34	4.77 **
L. corniculatus				
Density	1	114.95	0.10	4.31 *
Harvest (H)	4	163.58	0.08	3.18 *
Soil (S)	1	43.83	0.22	9.18 **
H x S	4	162.67	0.09	3.56 **
F. pratensis				
Density	1	185.89	0.03	12.83 ***
Harvest (H)	4	160.40	0.00	2.12 †
Soil (S)	1	39.60	0.01	2.42
H x S	4	160.12	0.00	1.95
P. lanceolata				
Density	1	197.60	0.05	1.28
Harvest (H)	4	161.91	1.34	31.82 ***
Soil (S)	1	42.20	1.21	28.81 ***
H x S	4	161.33	1.01	23.99 ***
A. millefolium				
Density	1	197.20	0.01	0.89
Harvest (H)	4	161.88	0.02	2.05 †
Soil (S)	1	42.21	0.24	20.18 ***
H x S	4	161.30	0.02	1.45
P. vulgaris				
Density	1	162.19	0.35	29.24 ***
Harvest (H)	4	163.10	0.16	13.79 ***
Soil (S)	1	43.83	0.00	0.06 †
H x S	4	162.29	0.07	5.48 ***

‡ The number of individual plants per microcosm at each harvest (Density) is included to account for density dependence in the response variables.

† = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

df = numerator degrees of freedom, DF_{den} Kenward-Roger adjusted denominator degrees of freedom (of error term), F = F-variance ratio.

Chapter 2

"The more clearly we can focus our attention on the wonders and realities of the universe about us, the less taste we shall have for destruction."

– Rachel Carson

Chapter 2

Soil Biodiversity Affects Ecosystem Multifunctionality

(Unpublished work)

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Summary

It is now well-established that anthropogenically induced biodiversity loss is occurring on a global level and there is increasing evidence that it will have severe consequences on the functioning of ecosystems. Earlier work has shown that the loss of soil biodiversity threatens ecosystem multifunctionality and ultimately sustainability. However, the limited analytical scope and duration of previous work limit our understanding of how soil biodiversity loss affects ecosystem multifunctionality over longer periods of time. Additionally, more recent publications have suggested that the interpretation of these previous results depend on the method used to calculate and analyze multifunctionality. To better understand the links between soil biodiversity and multifunctionality we established soil communities from differently managed agricultural soils and differently manipulated diversity levels and tested their effects on six ecosystem functions in grassland-ecosystem microcosms. We more than doubled the duration of the experiment in comparison with previous studies to test if reported positive correlations between soil biodiversity and ecosystem multifunctionality decreased or increased when the soil and plant communities were given a longer time to establish. We also used a novel approach to calculate ecosystem multifunctionality by utilizing multiple thresholds in addition to analyzing the various functions individually. This allowed us to eliminate weaknesses affecting other approaches pointed out in recent publications. Our results confirm previous studies showing that the loss of soil biodiversity has negative effects on the ecosystem functions of plant productivity, plant diversity, decomposition and soil nutrient uptake, retention, and cycling both when looked at individually and also when combined into a single value of multifunctionality. We suggest that the multiple threshold approach to multifunctionality could be used as a new standard to allow better comparisons with future biodiversity–ecosystem functioning experiments. The new approach also indicated that the samples from an organically managed

agricultural field site had higher levels of multifunctionality across the full range of ecosystem functioning thresholds than samples from two differently managed fields, suggesting that they were less sensitive to biodiversity loss. However, more replicates for the various soil management practices would be required to confirm this finding.

Keywords: community ecology, biodiversity–ecosystem functioning, multifunctionality, nutrient cycling, nutrient retention, primary production, soil biodiversity, soil degradation.

Introduction

There is an increasing body of evidence showing a link between soil biodiversity and the functioning of ecosystems (Brussaard 1997, Wall and Lynch 2000, Hooper et al. 2005, Eisenhauer 2011, Bradford et al. 2014). However, there has been debate within the scientific community about how best to assess the effects of biodiversity loss on ecosystem multifunctionality (Zavaleta et al. 2010, Bradford et al. 2014, Byrnes et al. 2014). As we are facing an increase in anthropogenic land management, it will be of critical importance to understand how land use and management intensity result in the depletion of soil biodiversity and how the changes in biodiversity affect ecosystem multifunctionality. Several studies have shown that soil biodiversity decreases with land-use intensification (cite). However, it is still poorly documented whether the effects of such differences in soil biodiversity between fields managed under different intensities cascade over to cause changes in ecosystem multifunctionality. Therefore, there is a rapidly growing need to investigate the effects of soil biodiversity and land management intensity and its impact on ecosystem multifunctionality. Such knowledge is required for a consensus among scientists on how to interpret, predict and perhaps prevent the impacts of biodiversity loss on the functioning of ecosystems (Wall et al. 2010).

Here we followed the methodology of Wagg et al. (2014) using a sieving series to create a soil biodiversity gradient from a highly diverse soil (a 5000 μm sieve) to a low-diversity soil (sterilized soil) that we inoculated into a sterilized substrate to test the effect that soil biodiversity had on a suite of ecosystem functions. Creating a soil biodiversity gradient using sieves decreasing in size removes species based on body size, which simulates systems with decreasing functional guilds rather than just decreasing species richness alone. This is useful for analyzing the effects of a simplification of the overall functional diversity of the soil community. The success and repeatability of the methodology of creating a soil

biodiversity gradient and simulating the loss of major functional guilds was confirmed in this and previous studies through fungal and bacterial DNA length-polymorphism analyses of the resulting soil communities (Wagg et al. 2014). To explore the interaction between soil biodiversity and land-use history/management intensity, the soil used to prepare the biodiversity-gradient inocula was collected from three different fields managed using either organic, conventional agricultural practices.

After establishment, the ecosystem functions of plant primary productivity, plant diversity, litter decomposition, carbon sequestration, soil nutrient uptake (P), and soil nutrient availability (N) were assessed. The experiment lasted for more than one year and included five plant-community harvests to test how ecosystem functioning was affected after a longer period of time. We assessed not only the effect of soil biodiversity on multiple ecosystem functions over time but also how this effect may vary across a sample of land management practices. We analyzed the trends of all ecosystem functions separately and together as a single score of multifunctionality and with a multiple threshold approach. This allowed us to see how the effect that soil biodiversity had on ecosystem functioning in our systems changed as the level of what is considered efficient functioning changed. An advantage of the multiple thresholds approach is that it maintains the value of each function in the final calculation of multifunctionality and prevents function substitutability, a problem that can occur when ecosystem functions are simply averaged (Bradford et al. 2014, Byrnes et al. 2014, Allan et al. 2015).

In summary we sought to answer the following questions: 1) how do multiple ecosystem functions change along a soil biodiversity gradient over a one year period? 2) How does the management intensity from where the soil inocula were sourced affect multiple ecosystem functions? 3) How does the measure of multifunctionality affect the outcome of the analysis of biodiversity–ecosystem functioning relationships.

Materials & Methods

Experiment Housing and Setup

Miniature self-contained cylindrical growth chambers (EcoTubes) (23 cm diameter x 34 cm height) were used as the experimental housing unit for this work as they provide a controlled and isolated environment where incoming pressured air and water are passed through hydrophobic (0.2 μm pore size) and hydrophilic (0.22 μm pore size) filters (all Millex®-FG_{50}; Millipore Corporation, Billerica, USA), respectively. This design minimizes greenhouse-borne microbial contamination, allowing the effects of manipulated microbial communities within each EcoTube to be more efficiently analyzed and their effects to be more accurately attributed. A detailed description of these EcoTubes can be found in van der Heijden *et al.* (2015). To better ensure the sterility of the microcosm environments, all EcoTube components were sterilized by autoclaving at 120 °C for a minimum of 20 minutes, with the exception of the Plexiglas tops and the polyvinyl chloride bottoms (see photo in Fig S1). As these parts of the EcoTubes would deform when autoclaved, they were sterilized through a process of a 20 minute submersion in 0.5% hypochlorite followed by a 70% Ethanol with Tween 20 bath before being immediately placed in the laminar-flow hood to air dry. The soil and plant communities were also planted under these same sterile conditions in an effort to minimize contamination.

Soil substrate and inocula

The bottom of each EcoTube was filled first with a 1 cm layer of quartz stones (approximately 1 cm diameter) before being covered with a propyltex screen (0.5 mm mesh size; Sefar AG, Heiden, Switzerland) to better accommodate drainage of excess water. As a sterilized substrate 5.5 kg (dry mass) of a 50/50 field soil/quartz sand mix sieved through a 5

123 mm mesh and autoclaved (120 °C for 90 minutes) was added on top of the screens in each
124 EcoTube. All soil for this sterilized substrate came from a natural grassland near the
125 Agroscope Reckenholz research station in Zürich, Switzerland (47° 25’ 38.71’’ N, 8° 31’
126 3.91’’ E). It was inoculated with soil collected from three agricultural fields with similar soil
127 structures but different management histories.

128 We used soils from three different management practices; “organic”, “conventional”
129 and “intensive” to inoculate the microcosms with various soil communities. These three soil
130 communities were used to better generalize our results across a gradient of site-specific land-
131 use histories and characteristics. The three soil communities reflect a gradient from less
132 intensive (“organic”) to intermediate (“conventional”) to intensive agricultural management
133 providing the opportunity to test whether there are indications that land-use intensity affects
134 the ability of soil communities to provide ecosystem services. The first two soils came from
135 the so-called DOK experimental field site in Therwil, Switzerland (47° 30' 8.9964” N, 7° 32'
136 21.8292” E). The DOK experiment was designed to assess different agricultural management
137 practices, such as various fertilizer practices, on various ecological and agricultural
138 characteristics of plots (see Mäder et al. 2002 for details). For the present study, soil was
139 collected from four plots where the management practice was the addition of organic
140 fertilizer (Site A, organic) and from another four plots where the management practice was
141 addition of mineral fertilizer (Site B, conventional). The third soil was sampled from a field
142 in Freiburg, Germany (47° 58' 26.058” N, 7° 46' 31.5336” E) that had been continuously
143 planted with the same crop (maize) for over 10 years (Site C, intensive). Details about soil
144 characteristics of each of the three sites are provided in Appendix S2 in Supporting
145 Information.

146 At all three sites soil was collected using four transects, one meter apart per plot,
147 coring soil every four meters. Soil cores were mixed per site and homogenized by sieving

through a 5 mm sieve. 250 g of fresh soil from each of the different sites was further processed by wet sieving through a series of decreasing mesh sizes using 1L dH_2O to create four levels of soil community inoculum treatments containing species with body sizes < 5000 µm (5 mm, no sieving), < 100 µm, < 25 µm, and sterile inocula (created by autoclaving for 90 min at 120 °C). An earlier study from Wagg et al. (2014) demonstrated that this methodology successfully manipulates soil biodiversity and soil community composition. Body size is known to be a useful functional trait because it is directly associated with the metabolic rates, population density, generation time and food size of different soil organisms and thus can be used to form functional groups (Bradford et al. 2002). Furthermore, several studies have shown that agricultural intensification selects for small-bodied functional groups (Postma-Blaauw et al. 2010). To reduce the differences that sieving out the larger soil particles could render, soil material not passing through the sieves was collected, autoclaved and added to back into the inoculum. These four sieving treatments were replicated five times for each of the three management fields, resulting in 60 total inocula. A single inoculum was added to the sterilized substrate in each of the 60 EcoTubes and the entire soil contents were mixed well. The inocula addition amounted to only 5% of the EcoTube soil volume, as we were interested in isolating the effects coming from differences in the soil microbial community, not from nutrient differences among the source sites.

In order to later measure the soil function of decomposition, two 0.5 mm propyltex mesh litterbags (6 cm x 6 cm) were each filled with 1 g of dried *Lolium multiflorum*, sterilized by autoclave, and buried just below the surface of the soil substrate in each EcoTube.

Plant community

Each EcoTube was planted with 12 individuals of the grass *Lolium perenne* and 12 individuals of the nitrogen-fixing legume *Trifolium pratense*, along with two individuals each of *Achillea millefolium* (forb), *Festuca pratensis* (grass), *Lotus corniculatus* (legume), *Plantago lanceolata* (forb), and *Prunella vulgaris* (forb), for a total of 34 individual plants per EcoTube (Lauber et al. 2012). These species commonly coexist in natural Swiss grasslands. Moreover, *T. pratense* and *L. perenne* are the two main species that commonly co-occur in managed grassland (e.g. permanent grassland or as fodder crops within crop rotations) in Switzerland. Additionally, *T. pratense* and *L. perenne* are model species belonging to different plant functional groups (legumes and grasses) and known to respond differently to soil biota: legumes depend heavily on associations with their soil biota for increased performance while grasses are less dependent on associations with soil biota (Klironomos 2003, Wagg et al. 2011a, 2014). We included the five other plant species in the experimental communities at a lesser abundance because they commonly occur in managed grass-clover fields, and they also allow for a better assessment of diversity responses of the plant community.

Seeds of each species were surface sterilized by immersion in 2.5 % hyposodium chlorate for five minutes, then rinsing thoroughly in distilled H_2O. Surface-sterilized seeds were then plated onto 1% Agar in Petri dishes to germinate. In order to ensure that the seedlings of all species were at the same stage of development when planted, the seed germination process was staggered so that each species exhibited the presence of cotyledon(s) or radicle when transplanted. Seedlings were planted into one of 34 evenly spaced and randomly selected positions in the inoculated substrate of each microcosm. These experimental communities were set up over eight days and the day on which each was set up was used as a blocking factor in the subsequent analysis of variance (ANOVA).

Once planted and sealed, all EcoTubes were placed into a greenhouse compartment where natural light was subsidized by 400-W high-pressure sodium lamps in order to maintain an environment of 16 h / 25 °C days and 8 h / 16 °C nights with a light level above 300 W/m2. Twice weekly, the EcoTubes were watered on an individual basis with dH_2O to maintain gravimetric soil moisture in the range of 10–20 %. However, since the greenhouse conditions maintain a constant environment, which does not reflect those found in nature which might allow for variation in plant species competitive interactions through time, we induced a variation in the watering regime to simulate an extended period without rain. The variation in precipitation was applied to all of the experimental communities at the same time by withholding watering for 10 days beginning five and a half weeks before each harvest. The plant communities were grown under these conditions for a total of 55 weeks.

Data collection

Over the 55-week growing period plant individuals were cut at 5 cm above the soil surface every 11 weeks to simulate the regular procedure of harvesting in agricultural grasslands which is common practice in Switzerland and other western/central European countries. Plants were harvested from EcoTubes according to the same schedule in which they were planted. Plant individuals were counted and separated by species, dried at 65 °C and the biomass weighed. For each harvest we calculated the total biomass (net primary productivity = NPP), and Shannon diversity index of the plant community (the sum of the biomass proportion of a species times the log proportion).

At each harvest one of the *L. multiflorum* litter bags was removed, the space was filled with a new, sterilized and labelled litter bag, and then the removed bag was washed clean of soil, dried at 65 °C, and the remaining litter biomass was extracted and weighed. Six randomly spaced soil cores of a 1.7 mm diameter and the full depth of the soil substrate

(approximately 20 g each) were taken during the harvest. Soil core samples were mixed well and divided into separate sterile containers for later analysis.

At the final harvest the roots were removed from each microcosm and cleaned of soil before being processed so that they could be assayed for arbuscular mycorrhizal fungal (AMF) colonization. Roots were cut into 2–5 cm fragments before a subsample was washed in 10 % KOH for 30 min and then stained with 0.05% (w/v) trypan blue in lactoglycerol. AMF colonization was then quantified under a microscope following the method outlined in McGonigal *et al.* (1990) scoring each for the absence/presence of AMF structures using 100 transects.

At 36 and 18 hours before each harvest each EcoTube was injected with 40 ml of ^{13}C labeled CO_2 (99 %) gas and sealed for one and two hours, respectively. This was done to get a measure of the atmospheric carbon fixation and belowground storage efficiency of the soil communities in each EcoTube at the end of the experiment. Soils samples to be analyzed for ^{13}C content were frozen at -20 º C and lyophilized following methodologies recommended by Krab *et al.*(2012).

At the completion of the experiment soil phosphorous (P) was measured (AAE10, AAE 1:10, P mg/kg) to analyze the efficiency of the soil to process and therefore facilitate plant uptake of excess soil P. Soil ammonium (NH4+) and nitrate (NO3-) concentrations were also analyzed at the end of the experiment and combined together as a single measure of the mineralization, nitrification and symbiotic fixation of soil N (Tate 1995).

Molecular assessment of soil communities

Following each harvest, DNA was extracted from 500 mg of combined and homogenized soil samples from each EcoTube using FastDNA® SPIN Kits for Soil (MP Biomedicals, Switzerland). The extracted DNA was quantified using a Quant-iT^{TM}

PicoGreen® (Molecular Probes, Eugene, OR) on a luminescence spectrometer (Perkin Elmer, LS 30, Rotkreuz Switzerland). All samples were then diluted to 10 ng / µl and used as DNA template in PCR reactions using the primers bRISArev and bRISAfor (FAM-labelled), targeting the 16s rDNA region with the cycling conditions and reagent concentrations outlined in Hartmann *et al.* 2005) and Wagg *et al.* 2014) for amplifying the bacterial community (see Hartmann *et al.* 2005 for full primer sequences).

The fungal DNA was amplified using primers fRISArev and fRISAfor (FAM-labelled) targeting the region ITS1-5.8S-ITS2 following the reagent concentration and cycling conditions outlined in Schneider *et al.*(2010). 12 µl HiDi-Formamid and 0.2 µl MapMarker® 1000 (BioVentures, Murfreesboro, TN) were mixed with two µl of the PCR products as the size standard and subject to fragment analysis in an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Run conditions were set to injection time of 30s at 1.5 kV and 10 s with a run time of 3000 s at 10kV. GeneMarker 1.91 genotyping software (SoftGenetics LLC, State College, PA) was used to characterize the unambiguous peaks of the amplified DNA fragments and the relative migration units were used as operational taxonomic units (OTUs). Peak intensities over the threshold value of 200 units and between 193 and 800 base pairs for fungi and > 800 florescence intensity with 150–600 base pairs for bacteria were scored as relative florescence units.

Data analysis

All statistical analyses were completed using R software (version 3.0.0; The R Foundation for Statistical Computing 2013), including the package ‘vegan’. In all analyses significance was determined as a type I error of $\alpha < 5$ %. Linear models were used to assess the variation in all ecosystem functions individually as a response to the experimental block, the field site from where soil inoculum was collected, and the soil sieving treatment, before

finally fitting the interactions of the field with sieve (analyzed as a factor) in ANOVA. To better isolate the effect of land-use intensity only, without the interactions and effects of the sieving treatment, we assessed the differences of all ecosystem functions among the different management field sites in the full soil (5000 µm) treatment only. The ecosystem function soil N was square-root transformed to scale the variation in residual error to better fit the models assumptions.

The data collected at each harvest for plant net primary production (NPP), plant diversity (*H'),* litter decomposition, and carbon sequestration (shifted to positive values for easier interpretation) were averaged over time to create a single functioning value for each measure. In contrast, the measure for excess soil phosphorus concentrations (P) and soil nitrogen availability (NH4 + NO3) (N) were taken only at the final harvest because measurement entailed destructive sampling techniques. The averaged values of the first four functions and the values of the additional two functions, i.e. all six ecosystem functions, were combined into a single value of ecosystem multifunctionality that we analyzed as a function of sieve size, i.e. resulting soil biodiversity. To do this, all individual functions were transformed so that they had the same average (0) and standard deviation (1), and then combined to create a single principle-component variable, the multifunctionality index (z-score). Higher values of this multifunctionality index represent more efficiently functioning ecosystems. Excess soil P concentrations were inverted for all multifunctionality analyses such that lower soil P concentration represent an increased ability of plants to utilize soil P; a well-known soil process mediated by soil microbes (Richardson and Simpson 2011). As an additional measure to analyze how our treatments affected the multifunctionality of our microcosms, all ecosystem functions were looked at with a holistic approach using a function developed based on the R package 'multifunc' by Byrnes et al. (2014). This method to assess multifunctionality is based on a multiple threshold approach by analyzing the effects of a

range of thresholds, or percentage of the maximum observed for each function, rather than setting a single threshold for what is considered functioning (Zavaleta et al. 2010, Byrnes et al. 2014). Multiple slopes illustrating the relationship between our soil biodiversity gradient and the number of ecosystem functions that were greater than or equal to the full range of thresholds from 0% to 100% were plotted. According to Byrnes et al. (2014), analyzing the changes in these slopes allows for more multifaceted conclusions that are adaptable to other experiments versus what can be done with results drawn from evaluating the statistical state of any single threshold slope alone. This methodology produces data showing the maximum effect of diversity (the greatest slope) as well as the threshold of analysis where this maximum slope is achieved (i.e., where diversity has the strongest effect on multifunctionality) along with a suite of other system multifunction-related diagnostics (Table 3).

Assessment of soil biodiversity loss

Soil fungal and bacterial communities were assessed through molecular methods (see above). The number of RISA peaks (OTUs) that came out of the RISA from each harvest were averaged together to create a measure of fungal and bacterial richness and plugged into our ANOVAs. AMF presence per 100 observations of root samples was used as a measure of AMF colonization.

Results

Sieving and site effects on soil microbial community structure

Our analysis of the richness of fungal and bacterial community OTUs in conjunction with the AMF colonization assays show that soil biodiversity was consistently degraded along the sieving continuum (Fig 1). The AMF presence in roots showed the greatest decline

321 in colonization occurring from the 100 µm down to the 25 µm treatment (from 77 % to 20 %
322 of the richness of the 5000 µm treatment) (Fig 1A). Fungal OTU richness decreased with
323 decreasing sieve size, with the most extreme drops in richness between the 25 µm and
324 sterilized soil treatments (from 78 % to 55 % of the richness detected in the 5000 µm sieved
325 communities) (Fig 1B). There were no overall differences among the three field sites in the
326 full soil treatment for both AMF presence ($F_{2,6} = 2.60$, $p = 0.154$) and fungal OTU richness
327 ($F_{2,6} = 1.15$, $p = 0.378$).

328 Bacterial OTU richness generally decreased with decreasing sieve size except from
329 the largest to the second largest size. The greatest change occurred between the 25 µm and 0
330 µm treatments (Fig 1C). Bacterial OTU richness did not significantly differ among the three
331 field sites in the full soil treatment ($F_{2,6} = 1.59$, $p = 0.280$).

332 When we combined all three measures of the soil community into a single normalized
333 index of soil biodiversity by averaging the standardized scores (z scores) of all ecosystem
334 functions, a generally decreasing overall soil biodiversity gradient was apparent. Thus, AMF
335 and fungal richness compensated for the non-conformity in bacterial richness in the overall
336 soil biodiversity measure in the full soil treatment (see appendix S3).

337

338 Sieving and site effects on plant communities

339 Removing the soil community with progressively smaller sieves yielded lower net
340 primary productivity of the grassland plant communities with the exception of the step down
341 from the 5000 µm to 100 µm treatments (Fig. 2A). Analyzing the plant functional groups
342 (grasses, legumes, and forbs) separately showed that there was a general trend of the legumes
343 and forbs making up a smaller proportion of the total plant biomass as well as their biomass
344 declining independently with decreasing sieve size. In contrast, the proportion of the biomass
345 that was made up by grasses increased with decreasing sieve size (Fig 2A). The grasses alone

supported most of the total community biomass in the lower soil biodiversity treatments, whereas the NPP of the grasses in the 5000 μm treatment with the most complete soil community was actually less than in the 100 μm treatment.

The negative effect that increasing soil community diversity, expressed in terms of sieve size, had on the NPP of grasses coupled with the positive effect that it had on the NPP of forb and legume species in sum yielded a positive relationship between soil biodiversity and plant diversity (Fig. 2B). However, NPP was not significantly different when we analyzed it among the three field sites in the full soil treatment only ($F_{2,6} = 1.22$, $p = 0.360$), while plant diversity was found to be marginally higher in soil from the two less intensively managed sites ($F_{2,6} = 4.79$, $p = 0.057$).

Sieving and site effects on decomposition, carbon storage, and soil nutrients

Litter decomposition and plant-available N were positively correlated with soil biodiversity while insoluble soil P showed the opposite relationship, with increasing values as sieve size decreased (Fig 2 C–F). In particular, soil P was strongly negatively correlated with AMF colonization ($r = -0.46$ – see full correlation table in Appendix S4, and plot in Appendix S5). Soil biodiversity did not show a clear effects on soil carbon storage; however, soil C was significantly negatively correlated with the proportion of the NPP made up by grasses ($r = -0.46$), while soil C was significantly positively correlated with the proportion of NPP made up by legumes ($r = 0.48$; see raw correlation plots in Appendix S5A–C). When the data for litter decomposition, nutrient storage, excess nutrient uptake, and plant-available nutrients in the full soil treatment were compared among the three source field site from which the soil inocula were collected, no clear patterns were detected (decomposition: $F_{2,4} = 3.57$, $p = 0.129$, C: $F_{2,6} = 0.089$, $p = 0.916$, P: $F_{2,6} = 0.182$, $p = 0.838$, N: $F_{2,6} = 0.304$, $p = 0.748$).

Sieving and site effects on ecosystem multifunctionality

When all effects were analyzed concurrently to gain a single measure of ecosystem multifunctionality, an overall decreasing trend was found along the decreasing sieve-size gradient from 5000 μm to 100 μm and then to 25 μm (52 % then 7 % of the full soil respectively), with a further decrease from the 25 μm to 0 μm (sterilized) soil treatment (almost two times less than the full soil) (see Fig 3A). The multifunctionality index decreased accordingly with decreases in realized soil biodiversity (see Fig 3B), confirming that sieving successfully manipulated soil biodiversity. There were no significant differences in ecosystem multifunctionality among the three fields in the full soil treatment ($F_{2, 4} = 0.307$, $p = 0.752$) (Fig 3A-B).

Using the method of Byrnes et al. (2014) to analyze the relationship between soil biodiversity (i.e. here its proxy sieve size) and ecosystem functioning, we found that the threshold at which diversity began to have an effect on multifunctionality (Tmin) was much lower in the systems with inocula from the intensively managed site (13 % of maximum functioning) while the organically and conventionally managed soils had more similar higher thresholds (86 % and 83 % respectively) (Fig. 4). The upper threshold beyond which diversity had no effect on multifunctionality (Tmax) was also lower in the intensive field soils versus that of the conventional field. Notably, our current analysis did not successfully determine the upper threshold for the systems with inocula that came from the organically managed fields (Table 3). Diversity was found to have the strongest effect on multifunctionality (Tmode) at just 47% of maximum functioning in the intensive soils, and this effect was actually negative. In contrast, for the conventional and organic soil sources, soil diversity had the strongest effect at 85 % and 96 % of maximum functioning, respectively, and the effect was positive for both (plotted in Appendix S6).

Discussion

The results of this study demonstrating the positive relationship between soil biodiversity and multiple ecosystem functions parallel those of an earlier study by Wagg et al. (2014). Because the present study differed in several aspects, in particular the much longer duration, broader generalizations are now possible based on the two studies. Both studies show that manipulations of soil biodiversity via sieving affect ecosystem multifunctioning in model systems regardless of duration, and finally but importantly, how ecosystem multifunctionality is assessed (Byrnes 2013 versus Bradford 2014). The new results also confirm trends found in other previous studies, elucidating the tie between soil biodiversity loss and significant decreases in multiple ecosystem functions (Bradford et al. 2002, Bonkowski and Roy 2005). Overall, the new results provide even stronger evidence of a link between soil biodiversity and ecosystem multifunctioning because we came to the same conclusions via three different angles of analysis.

Soil microbial community structure

The strong decrease in AMF colonization on the sampled roots in the interval between the 100 µm and 25 µm treatment (from 77 % to 20 % of the richness of the 5000 µm treatment) coincides with interval in which the average size of AMF spores lies, namely 40 µm (Marleau et al. 2011). In contrast to AMF and fungi, the generally smaller bacteria did not decline from the largest (5000 µm) to the second largest (100 µm) sieve size treatment. However, for both fungal and bacterial richness it must be considered that richness does not give the full picture of the structure of soil microbial communities. For example, although there is a slightly lower richness of bacterial OTUs in the full soil treatment, the diversity of

420 the functions that the particular bacterial species perform could be larger than in the 100 µm
421 treatment.
422
423 ## Plant productivity and diversity
424 NPP was slightly lower in the highest soil diversity treatment (5000 µm) than in the
425 next sieving size down (100 µm). However, NPP declined steeply and steadily down with the
426 lower soil diversity treatments. This pattern of variation in NPP results from a decline in the
427 biomass of legumes and forbs down the soil biodiversity gradient (~ 55 % of NPP in the full
428 soil but only ~2 % in the sterilized soil treatment), coupled with increases in the proportion of
429 grass biomass (~45 % in full soil and ~98 % in sterilized soil). The slightly lower NPP in the
430 full soil treatment as compared to the 100 µm treatment was also found by Wagg et al. (2014)
431 and can be explained by decreased ability of the grass species to compete as well against the
432 forbs and legumes as they could in the lower diversity soil treatments – i.e. their greater
433 biomass in the low-diversity soils made up a larger proportion of the biomass (see plant
434 functional group proportion bars in Fig 2A). This is in line with previous studies that
435 documented grasses maintaining NPP when soil biodiversity levels were reduced below
436 levels similar to our 100 µm sieve size treatment(Bradford *et al.* 2002; Wagg *et al.* 2014;
437 Pellkofer *et al. unpublished*). The dominance of the grasses in the low-diversity treatments
438 was stronger in both the Wagg et al. (2014) study and at earlier time points of our experiment
439 (results not presented here). In the present study, the additional half-year growing period
440 benefited the forbs and legumes such that they could constitute an increasingly larger
441 proportion of the NPP and that the correlation between soil biodiversity and total NPP could
442 develop more strongly. This can be likely explained by the continuing assistance that these
443 functional groups had from the soil community in obtaining nutrients in the systems and
444 being overall better competitors to the grasses, especially as the systems were not fertilized,

445 therefore they were more stressed in the later stages of the experiment. It is conceivable that
446 the frequently observed increase in plant diversity–productivity relationship in grassland
447 biodiversity experiments over time (Cardinale et al. 2007, Reich et al. 2012) is in part also
448 due to the development of plant–soil interactions over time (Zuppinger-Dingley et al. 2014).
449 The increasing downward slope of NPP from 100 μm to the lower sieve sizes makes
450 sense because due to the spore size of most AMF spores (>40 μm) the 100 μm mark is a
451 critical cut-off point for the benefits that AMF have been found to provide to plant
452 productivity and plant diversity (van der Heijden et al. 1998, Maherali and Klironomos 2007,
453 Wagg et al. 2011a). Without the symbiotic assistance from AMF we know that forbs and
454 legumes can be more easily outcompeted for resources by grasses (Marler et al. 1999, Wagg
455 et al. 2011b) leading to a dominance of grass biomass in the soils with the most depleted soil
456 biodiversity levels. Additionally, the direct and indirect effects of the likely presence of both
457 soil diversity levels probably is an important factor underlying the increased plant community
458 diversity with higher soil biodiversity (Putten et al. 1993, Bezemer et al. 2010, Wagg et al.
459 2014). These positive effects on both plant biomass production and plant diversity is
460 something that can be viewed as beneficial ecosystem functions, especially for agricultural
461 systems using mixed-cropping designs.
462

463 ## Litter decomposition, and nutrient storage, uptake, and availability

464 In line with previous studies (Wall and Moore 1999, Wagg et al. 2014), soil
465 biodiversity loss was associated with a reduction in the decomposition of organic matter. This
466 makes sense as the efficiency of organic matter decomposition has been well-documented as
467 being directly tied to the presence and functional diversity of soil organisms (Heemsbergen et
468 al. 2004, Bonkowski and Roy 2005). Compared with the earlier study of Wagg et al. (2014),

469 doubling the duration of the experiment made the downward slope of decomposition with
470 decreasing soil biodiversity even stronger.

471 To explain potential underlying mechanisms of ecosystem functioning, we compared
472 our soil C sequestration results to other ecosystem function responses (see full correlation
473 table in Appendix S4). Soil C sequestration was negatively correlated with the grass
474 proportion of NPP but positively correlated with the proportion of biomass made up by
475 legumes along the soil diversity gradient. This is a logical tie in light of previous studies that
476 have documented the link between the presence of legumes and increases in soil C pools (De
477 Deyn et al. 2009).

478 Soil P was most negatively correlated with AMF colonization of roots, emphasizing
479 that AMF facilitate more efficient use of soil P as has been shown by previous studies (van
480 der Heijden et al. 1998, Bonkowski and Roy 2005). Lower values of available P in the soil
481 can be considered a beneficial ecosystem function because loss of soil P, similar to loss of
482 soil N, through leaching can result in unwanted eutrophication and in addition reduces the P
483 held in the ecosystem (Tilman et al. 2002, Carpenter 2008). This is especially an issue in
484 agricultural situations where additional P is being applied in the form of fertilizer (Celardin
485 2003). Soils with healthy soil communities have been found to more efficiently mineralize
486 soil P into a more soluble form that plants can easily uptake. The lower P levels found in our
487 soil samples associated with higher soil diversity levels reinforce the likely occurrence of soil
488 microbes directly utilizing mineralized nutrients perhaps along with the combined effects that
489 the increased soil biodiversity has on plant productivity. In other words, a larger biomass of
490 more healthy plants is more efficient at acquiring mineralized P (Hooper and Vitousek 1998).

491 The decreased quantities of plant-available soil N in the form of ammonium (NH_4+)
492 and nitrate (NO_3-) found with decreased soil biodiversity in our model system demonstrate
493 the role that soil microbes play in efficiently mineralizing and nitrifying the various forms of

494 N put into a system. This link is especially important to consider because it is known that the
495 efficiency of soil N immobilization in plants has been decreasing while at the same time the
496 input of synthetic N in the form of fertilizers and manures has been increasing in an effort to
497 increase crop yields (Vitousek 1997). Maintaining soil biodiversity could help to combat N
498 leaching and pollution that is likely to increase in intensity with current standards in
499 agricultural management practices.
500

501 Multifunctionality

502 When all of the measured functions were compiled into a single multifunctionality
503 score, it was not surprising that multifunctionality showed the same response as the functions
504 showed individually, i.e. of a decrease with decreasing sieve sizes. The effect of soil
505 biodiversity on multifunctionality in our model systems was further reinforced when we
506 compared the realized soil biodiversity index to multifunctionality (Fig 3B) and found a
507 strong negative correlation between realized soil biodiversity and multifunctionality. The
508 trend of decreasing multifunctionality with decreasing soil biodiversity in the present study
509 was even more pronounced than in the shorter study of Wagg et al. (2014), suggesting that
510 the effect that soil biodiversity can have on multifunctionality could possibly increase in
511 strength over time.

512 The threshold plots produced using the Byrnes methodology of analyzing
513 multifunctionality (Fig 4) show interesting differences between the three field sites from
514 which our soil inocula were obtained. The change in the number of functions per increase of
515 diversity level was larger for the field site with a management history of organic agriculture
516 when lower thresholds of functioning were considered. In contrast, the maximum slope of the
517 conventional and intensive soils increased at a slower rate as the thresholds of percentage of
518 what was considered “successful functioning” increased. When 50% of maximum

519 functioning and higher is set as the minimum for what is considered successful functioning,
520 we see that in the conventional soils biodiversity actually has a negative effect on
521 multifunctionality but in the organic and conventional systems the level of change remains
522 about the same with greater variability.

523 The output metrics from the same analysis also yielded interesting differences in the
524 effects of manipulated soil diversity among the different field sites. The minimum threshold
525 where diversity began to have an effect in the systems with the intensively managed soil
526 inocula were much lower than those with inocula from the organically or conventionally
527 managed sites – 13 % of maximum multifunctionality versus > 80% for the two less intensive
528 sites. However, when the threshold level of what was considered successful functioning was
529 raised, the multifunctionality of the field site with intensive management was negatively
530 affected by soil biodiversity and the maximum threshold at which soil biodiversity yielded
531 effects on multifunctionality was much lower than for the two other field sites with organic or
532 conventional management. The metrics output using the Byrnes et al. method also
533 interestingly showed that in the intensive soils the largest effect that increases in soil
534 biodiversity had on multifunctionality was actually a negative effect and it occurred around
535 50% of maximum functioning. In contrast, the strongest effect that changes in soil
536 biodiversity had on multifunctionality in the conventional and organic soils were positive and
537 found when only considering successful functioning to be above 85 % of the maximum.
538 These differences reinforce the message from Byrnes et al. (2013) that the conclusions that
539 can be drawn on how much of an effect soil biodiversity has on multifunctionality depend
540 heavily on which percentage of the maximum functioning is considered as the threshold.
541

542 ## Outlook

543 Picking apart the intricate web of interactions that make up the soil biodiversity and
544 ecosystem functioning relationship looms as an outstanding task for scientists. Based on the
545 results of this study we suggest that more work be done to investigate the specific soil
546 organisms and soil functional guilds associated with changes in ecosystem multifunctionality,
547 using the advancing technologies in soil molecular analysis tools that are becoming more
548 readily available. Tying specific groups of organism and critical thresholds of soil
549 biodiversity to ecosystem functioning can help to guide future management efforts to
550 maintain and improve soil biodiversity so that a wealth of ecosystem functions are sustained.
551 The results of our multiple-thresholds approach to analyzing multifunctionality
552 demonstrate that the link between biodiversity and ecosystem multifunctionality can be
553 interpreted differently based on soil management history differences of the samples as well as
554 which threshold of functioning is selected as the baseline for analysis. This suggests that our
555 understanding of how this link varies across land uses is in its infancy; therefore we
556 recommend more experiments be performed with greater site replication to create a larger
557 pool of data from which more accurate conclusions can be drawn.
558 We further suggest that future studies use our data and collect more from replicate
559 fields to further investigate the relationship between agricultural management practices, soil
560 biodiversity and ecosystem functioning. And although we did increase the duration of this
561 experiment by over double of previous work, based on the increases in the strength of the
562 relationship we found between soil biodiversity and ecosystem multifunctionality, we believe
563 that conducting future studies over an even longer period of time could better elucidate how
564 the temporally distant implications of soil biodiversity loss might change. Furthermore,
565 additional analyses looking at how these systems developed temporally would be helpful in
566 better elucidating the interactions of the soil community with the performance of ecosystem

567 functions. We take the temporal perspective on the biodiversity–ecosystem functioning
568 relationship in the next chapter of this dissertation.

569 Whether the individual functions were analyzed separately or concurrently the
570 ultimate result was the same: soil biodiversity loss significantly inhibits the functioning of
571 ecosystems. Further investigation into the precise nuances of the functional roles that specific
572 soil organism or guilds of organisms perform and how those guilds interact to improve or
573 inhibit the functioning of ecosystems is an obvious path forward in research to prevent
574 further degradation of the ecosystem functioning processes on which we rely.
575

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706

Tables

Table 1. ANOVA results for the effects of field source of soil inocula (Field) and soil sieving treatment (Sieve), and the interaction of the two variables on the presence of arbuscular mycorrhizal fungi (AMF) and fungal and bacterial richness. To account for differences arising from the experimental design, the planting and harvest day (Block) was fitted first in our model.

		AMF		**Fungal Richness**		**Bacterial Richness**	
	DF_{num}	Sum Sq	F	Sum Sq	F	Sum Sq	F
Block	7	2136	1.78	4521	9.00***	3026	1.42
Field	2	2019	5.87**	223	1.55	468	0.77
Sieve	3	36789	71.3***	1392	6.46**	9651	10.58***
Field:Sieve	6	2291	2.22·	117	0.27	1001	0.55
Residuals	*41*	*7048*	--	*2944*	--	*12462*	--

·P<0.06, * P < 0.05, ** P < 0.01, *** P < 0.001, DF_{num} = numerator degrees of freedom, Sum Sq = sum of squares, F = F-ratio.

716 Table 2. ANOVA results for the effects of field source of soil inocula (Field) and soil
717 sieving treatment (Sieve), and the interaction of the two variables on ecosystem functions
718 NPP, plant diversity, decomposition, C sequestration, insoluble soil P, available soil N, and
719 multifunctionality. To account for differences arising from the experimental design, the
720 planting and harvest day (Block) was fitted first in our model.

		NPP		Plant Diversity		Decomposition		C Sequestration	
	DF_{num}	Sum Sq	F	Sum Sq	F	Sum Sq	F	Sum Sq	F
Block	7	33.7	2.05	0.84	7.78***	0.05	2.14	106	7.52***
Field	2	10.8	2.29	0.14	4.53*	0.01	1.51	1.59	0.39
Sieve	3	43.7	6.20**	5.34	115***	0.04	3.77*	13.67	2.26
Field:Sieve	6	2.58	0.18	0.10	1.06	0.02	1.21	6.59	0.54
Residuals	*41*	*96.1*	--	*0.633*	--	*0.142*	--	*82.7*	--

		Excess soil P		Available soil N		Multifunctionality	
	DF_{num}	Sum Sq	F	Sum Sq	F	Sum Sq	F
Block	7	196	0.85	0.89	2.33*	2.64	5.53***
Field	2	39.9	0.61	0.001	0.03	0.19	1.38
Sieve	3	741	7.55***	2.67	16.4***	14.0	68.4***
Field:Sieve	6	25.1	0.12	0.33	1.00	0.13	0.31
Residuals	*41*	*1341*	--	*2.23*		*2.65*	

721 * P < 0.05, ** P < 0.01, *** P < 0.001, DF_{num} = numerator degrees of freedom, Sum Sq = sum of squares, F = F-ratio.
722

723 Table 3. Results from multifunctionality analysis showing the minimum threshold where
724 diversity begins to have an effect (Tmin), the upper threshold beyond which diversity has
725 no effect on multifunctionality (Tmax), the threshold where diversity has the strongest
726 positive or negative effect (Tmode), the strength of the slope where diversity has its strongest
727 effect (Rmode), and the percentage of maximum possible relative importance of diversity for
728 multifunctionality (Pmode) under each field management type (site A: organic, site B:
729 conventional, and site C: intensive).

	Tmin	Tmax	Tmode	Rmode	Pmode
Site A	86	NA	96	0.23	0.70
Site B	83	88	85	0.19	0.70
Site C	13	68	47	0.21	0.70

730

Figures

Fig. 1. Means ± SEM of the change in soil community measures along the sieving treatment gradient according to sieve size (5000–0 µm) for A) fungal and B) bacterial richness (all sampling time points), and C) AMF colonization (end time point only). Sieve is shown on the +1(to avoid mathematical errors with 0 µm)-log scale for a more realistic comparison of the size differences. Measures reflect both richness (fungi and bacteria) and abundance (AMF) of the established soil community. Lines highlight the general trend of the characteristics along the sieving gradient. Colored bars to the left correspond to the means and standard errors for each measure in the full soil treatment only from each field source (Site A - organic, Site B – conventional, and Site C – intensive).

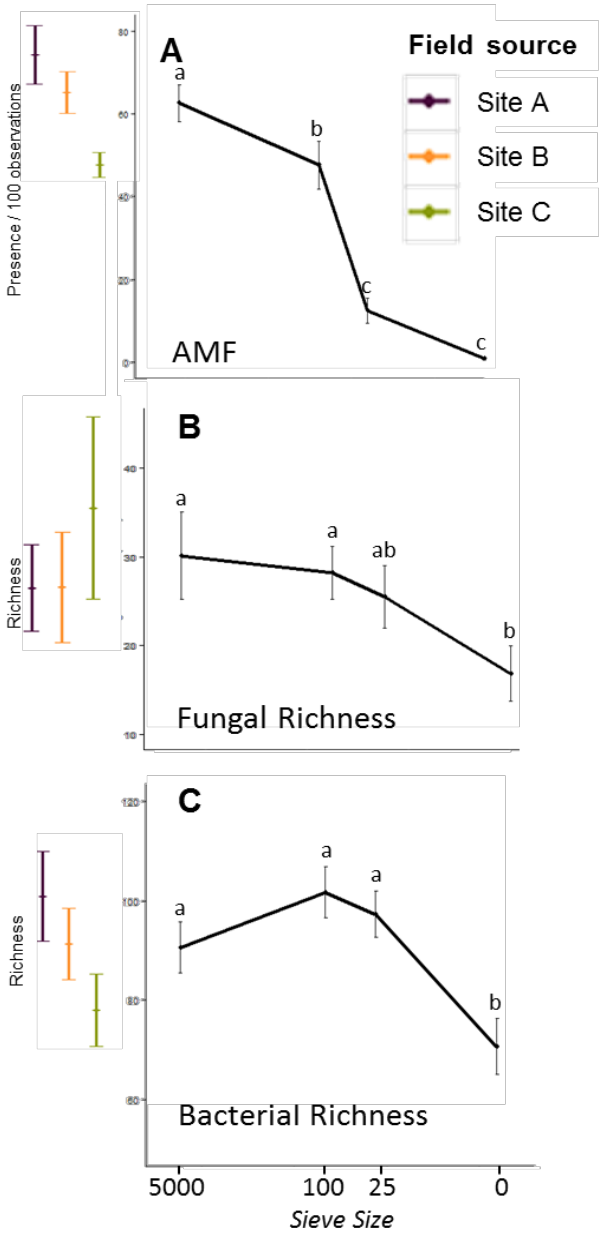
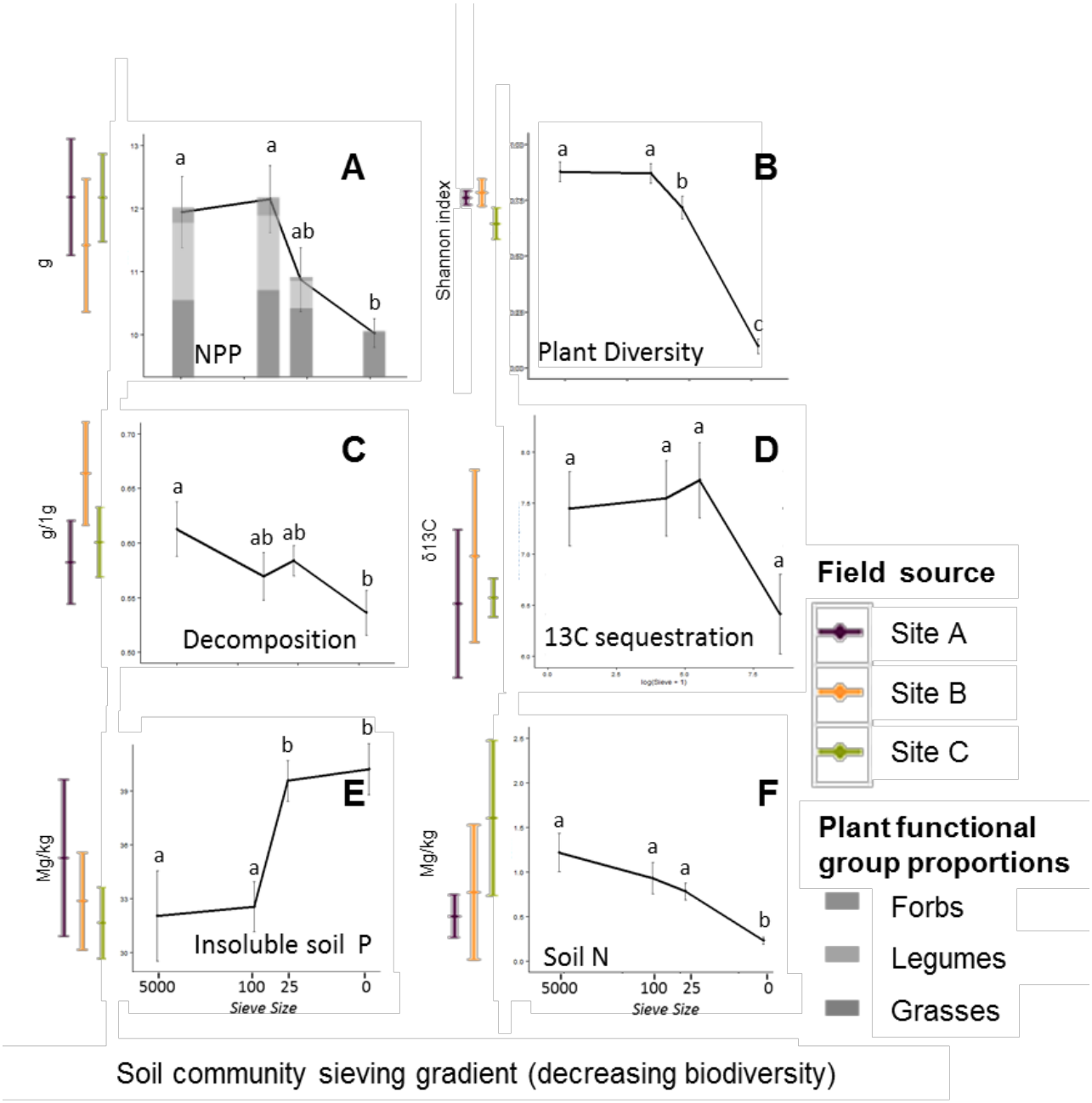


Fig. 2. Raw means and +/- SEM along the (log scale) sieving gradient for the ecosystem functions: A) net primary production (NPP), B) plant diversity, C) litter decomposition, and D) soil carbon sequestration (shifted to positive values for interpretation) as the mean values of all harvests, with E) insoluble soil P, and F) soil N availability as the mean values from the final harvest only (due to the destructive sampling method required). Colored bars on the left panel of each figure correspond to the means and standard errors for each ecosystem function of the full soil (5000 µm) treatment of the three separate agricultural management fields (Site A - organic, Site B – conventional, and Site C – intensive).



749 Fig. 3. A) Ecosystem multifunctionality index (z-score) of all ecosystem function measures
750 combined in relation to sieve size (µm) with lines showing linear model regression estimates
751 with standard errors and letters showing differences from model estimates. B) Ecosystem
752 multifunctionality index (z-score) of all ecosystem function measures combined in relation to
753 the realized soil biodiversity (z-score) with the linear model regression estimate. Colored bars
754 to the left correspond to the z-score means and standard errors the full soil (5000 µm)
755 treatment of the three separate agricultural management fields (Site A - organic, Site B –
756 conventional, and Site C – intensive).

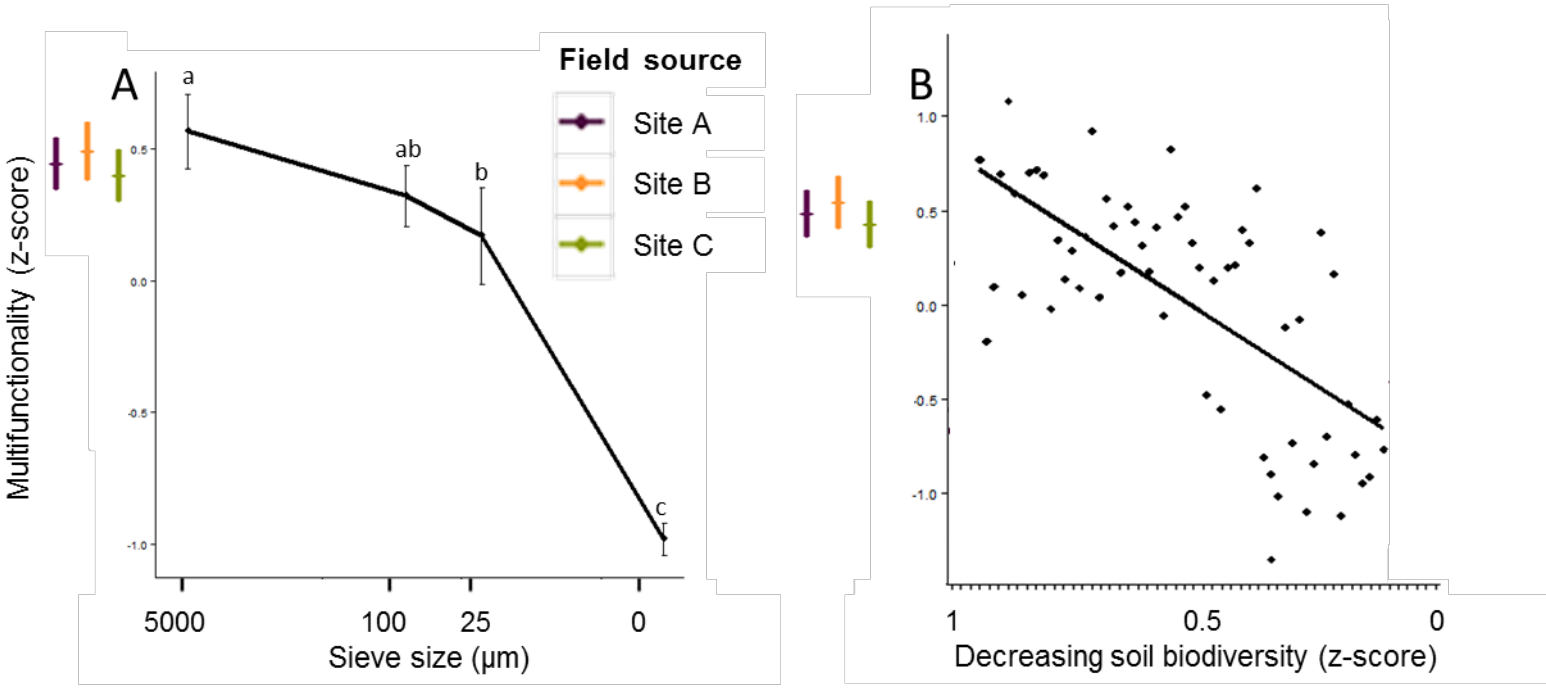
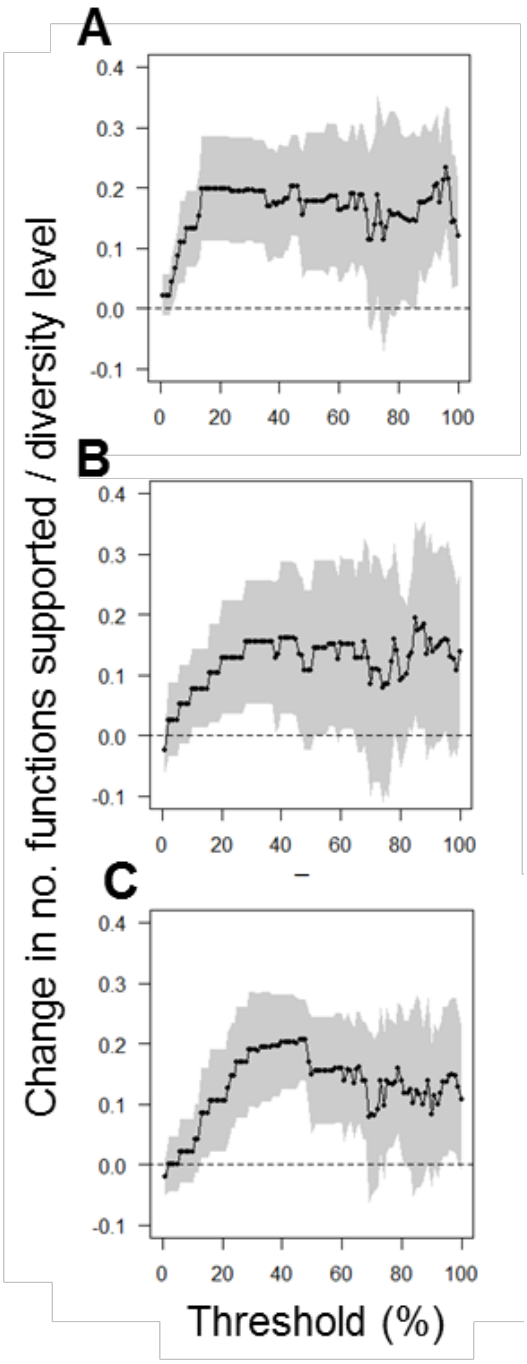
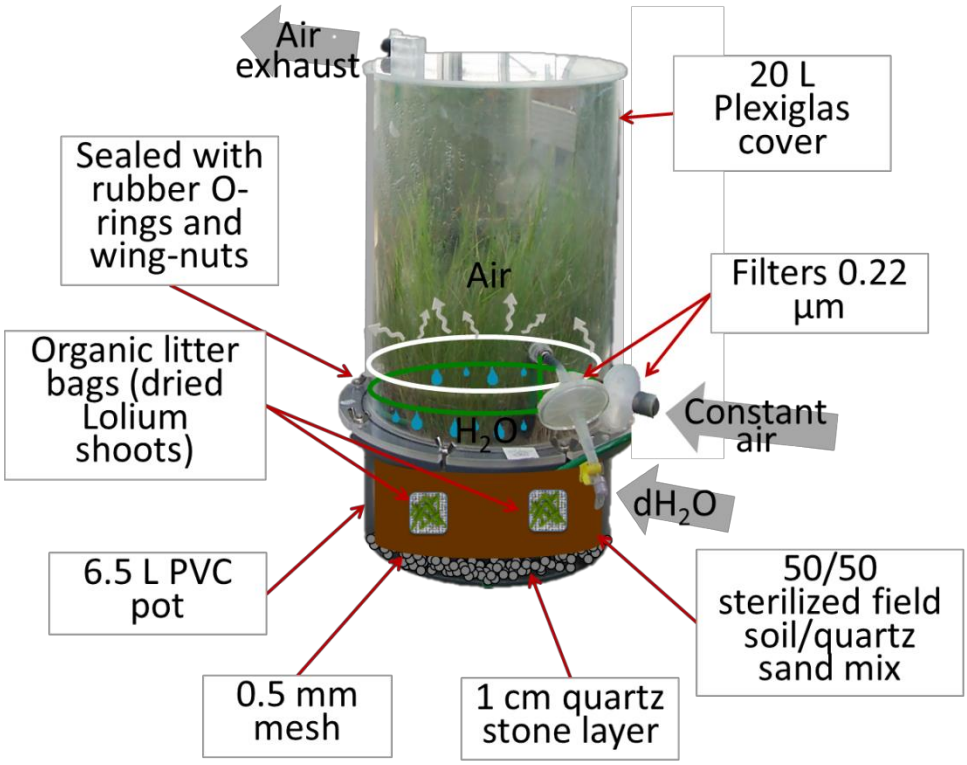


Fig. 4. Change in the number of ecosystem functions supported by soil biodiversity with increasing threshold (%) at which ecosystem functions are considered as a portion of the maximum ecosystem functioning for A) Site A – organic, B) Site B – conventional, and C)Site C – intensive management fields.



Supplementary Information

Appendix S1. EcoTube microcosms design.



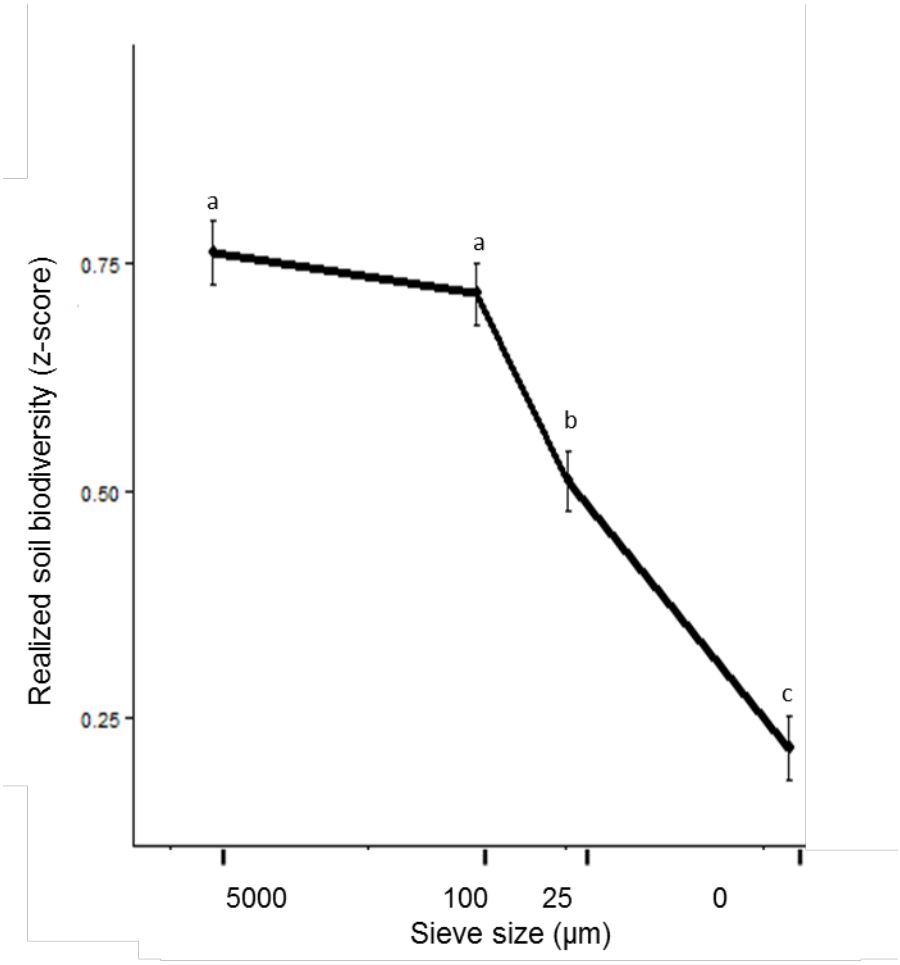
763 Appendix S2. Inocula soil history with initial soil properties analysis results. pH (pH),
764 $mg \cdot kg^{-1}$ of plant available potassium (K-Test) and magnesium (Mg-Test) content extracted
765 with ammonium acid-extraction, CO_2 and $CaCl_2$. Ammonium acetate-EDTA (pH 4.65) was
766 used to extract K, Mg, and Ca in $mg \cdot kg^{-1}$.

Term	Management	Site name & location	pH	K-Test *	Mg-Test *	K *	Mg *	Ca *
Site A, organic	organic fertilizer, "bio-Organic"	DOK† trial, Therwil, Switzerland	7.9	1.9	9.9	61.1	206	6228
Site B, conventional	mineral fertilizer, "Konventionell"	DOK trial, Therwil, Switzerland	7.4	2.0	10.4	59.7	202	6074
Site C, intensive	Intensive, 10-year maize mono cropping	Private farm, Freiburg, Germany	7.4	1.7	10.8	65.0	216	6533

* Results units are mg/kg
† Biologisch-dynamisch, organisch-biologisch and konventionell (DOK) trial. See www.fibl.org for more information about the study design (Mäder *et al.* 2002).

767

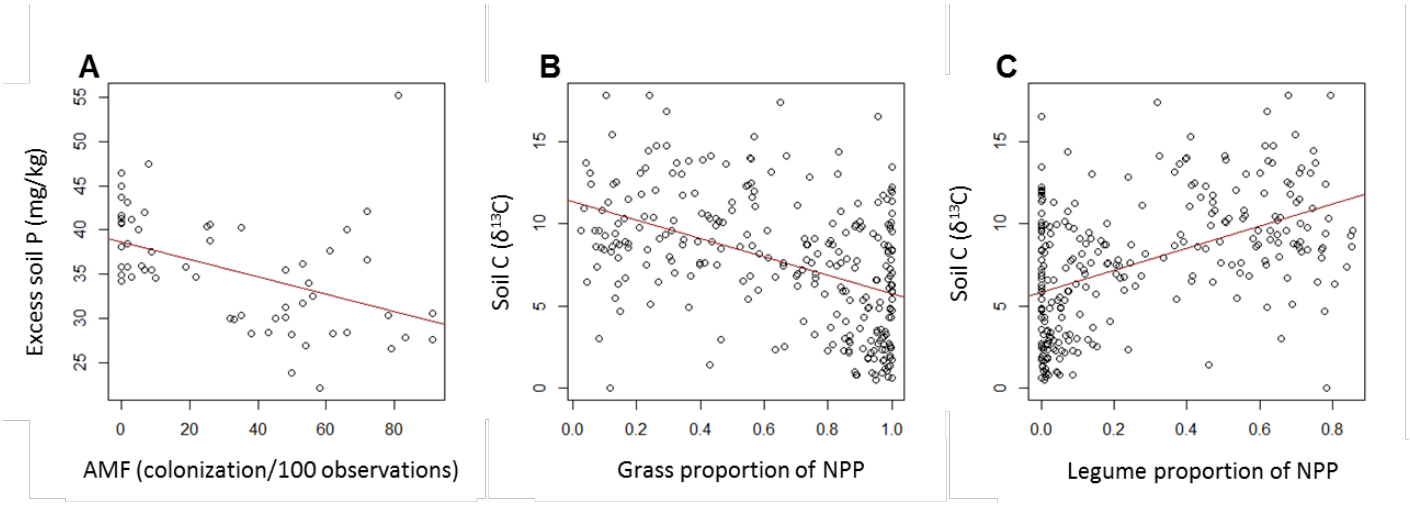
768 Appendix S3. Combined normalized value ± SEM soil community measures (fungal and
769 bacterial OTU richness from all sampling time points and AMF presence from the final
770 harvest only) along the sieving treatment gradient according to sieve size (log scale 5000-0
771 µm). Lines highlight the general trend of the characteristics along the sieving gradient.



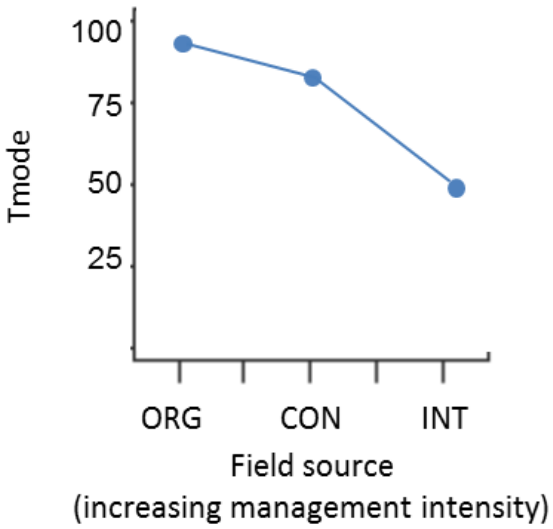
Appendix S4. Correlation matrix between all 12 variables of interest (nine ecosystem functions: NPP, grass, legume and forb proportion of total biomass, plant diversity, decomposition, C sequestration, excess soil P, and available soil N, as well as three soil community characteristics: AMF presence, fungal richness, and bacterial richness).

	NPP	Legumes	Grasses	Forbs	Plant Div	Decomp	Soil C	Soil P	Soil N	AMF	Fungi	Bacteria
NPP		0.264	-0.231	0.003	0.190	0.161	0.101	-0.262	0.323	0.488	0.126	0.085
Legumes	0.264		-0.975	0.417	0.789	0.122	0.480	-0.455	0.545	0.692	0.068	0.469
Grasses	-0.231	-0.975		-0.609	-0.851	-0.135	-0.458	0.461	-0.546	-0.777	-0.089	-0.461
Forbs	0.003	0.417	-0.609		0.664	0.114	0.154	-0.274	0.305	0.660	0.121	0.216
Plant Div	0.190	0.789	-0.851	0.664		0.138	0.419	-0.682	-0.715	0.637	0.263	0.339
Decomp	0.161	0.122	-0.135	0.114	0.138		-0.035	-0.059	-0.250	0.018	-0.052	0.109
Soil C	0.101	0.480	-0.458	0.154	0.419	-0.035		-0.187	-0.343	-0.173	0.032	0.332
Soil P	-0.262	-0.455	0.461	-0.274	-0.682	-0.059	-0.187		0.724	-0.455	-0.304	-0.160
Soil N	0.323	0.545	-0.546	0.305	-0.715	-0.250	-0.343	0.724		0.421	-0.195	0.124
AMF	0.488	0.692	-0.777	0.660	0.637	0.018	-0.173	-0.455	0.421		0.342	0.165
Fungi	0.126	0.068	-0.089	0.121	0.263	-0.052	0.032	-0.304	-0.195	0.342		0.152
Bacteria	0.085	0.469	-0.461	0.216	0.339	0.109	0.332	-0.160	0.124	0.165	0.152	1.000

Appendix S5. Plots of raw data showing correlation between A) excess soil P content (in mg/kg) (r= -0.46), and AMF colonisation/ 100 observations, B) the proportion of NPP made up by grasses with soil carbon content (in $\delta^{13}C$) (r =- 0.46), and C) the proportion of NPP made up by legumes with soil carbon content (in $\delta^{13}C$) (r = 0.48) with linear model regression estimates showing the correlation trends in red.



Appendix S6. The threshold percentage where diversity has the strongest positive or negative effect (Tmode), on multifunctionality, as calculated using the Byrnes multifunctionality function method, for each field management type shown by increasing intensity of land-use along the x-axis (ORG – organic > CON – conventional > INT – intensive).



Chapter 3

"When we try to pick out anything by itself, we find it hitched to everything else in the Universe"

– John Muir, 1911

Chapter 3

Soil Biodiversity Improves the Stability of Ecosystem Functioning

(Unpublished work)

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Summary

Global biodiversity loss is acknowledged as one of the greatest threats to the sustainability of ecosystem functions that maintain the ecosystem services upon which societies depend. Specifically, plant and soil biodiversity and community composition are known key factors in determining the performance of multiple ecosystem functions. However, it remains unknown whether soil biodiversity influences the functioning and stability of multiple ecosystem functions through time. Here, we manipulated soil biodiversity and tested its impact on four ecosystem functions, measured every two and a half months, over a period of one-year, in model grassland microcosms. We show that the loss of soil biodiversity negatively affected the mean performance, increased the temporal standard deviation, and reduced the stability of the performance of multiple ecosystem functions, including plant productivity, plant diversity, and litter decomposition. Higher soil fungal taxa diversity and stability in ecosystem functioning was associated with a greater negative covariance (asynchrony) among fungal taxa over time, suggesting that decreases in functioning of one fungal species are compensated by increases in other fungal species. These results indicate that soil microbial diversity and associated species asynchrony are important elements for the reliable provisioning of ecosystem services in the face of impending climate changes.

Keywords: Asynchrony, biodiversity and ecosystem function, community ecology, ecosystem stability, multiple ecosystem functions, nutrient retention, primary production, soil biodiversity.

Introduction

There is an ever-growing body of literature showing the links between biodiversity and the performance and stability of various ecosystem functions (Hooper *et al.* 2005; Loreau & de Mazancourt 2013). As such, there is consensus that losses of biodiversity negatively affects individual and multiple functions simultaneously (Hooper *et al.* 2005; Hector & Bagchi 2007; Isbell *et al.* 2011; Byrnes *et al.* 2013). There is also consensus that decreases in biodiversity can destabilize the functioning of an ecosystem (Tilman, Reich & Knops 2006; Cardinale *et al.* 2012; Hautier *et al.* 2015). This destabilization can arise through a reduction of asynchronous response to temporal environmental fluctuations by the different species that comprise the community. This is because the decline in the performance of one species is less likely to be compensated for by another species that would thrive under those same environmental conditions as the richness of the species pool declines (Isbell, Polley & Wilsey 2009; Loreau 2010; Hector *et al.* 2010).

However, much of the headway in understanding how biodiversity maintains the functioning of ecosystems has been largely based upon plant communities. Meanwhile, it is becoming increasingly apparent that the soil community is a crucial component underpinning the successful functioning we observe in aboveground communities. For example, recent studies have confirmed that changes in soil community diversity and composition are effective predictors of the performance of several ecosystem functions, including plant productivity, diversity, and nutrient cycling (Wagg *et al.* 2014; Bradford *et al.* 2014). However, our knowledge base in understanding how diversity and temporal fluctuations in the soil communities cause changes in the stability of ecosystem functions is lacking. This is crucial information, as it is becoming well understood that anthropogenic disturbance to soils through various mainstream land management practices, like large scale intensive agriculture, have drastic effects on the composition and diversity of soil organisms (Matson 1997). With

our ecosystems facing both a changing climate as well as ever-increasing anthropogenic pressures for productivity among other ecosystem services, developing a better understanding of how changes in soil communities can impact the stability of ecosystem functions over time is more critical than ever (Wall, Bardgett & Kelly 2010).

Here we address the hypotheses that 1) soil biodiversity loss alters the performance and temporal stability in multiple ecosystem functions, and 2) if soil biodiversity loss destabilizes ecosystem functions, than it is because soil biodiversity loss reduces the ability of species asynchronous responses to environmental fluctuations to occur in soil communities; as has been theorized and observed in plant communities; typically coined the "insurance" or "portfolio" effect (Naeem & Li 1997; Doak *et al.* 1998; Yachi & Loreau 1999; Hector *et al.* 2010). To test this we paired a soil sieving protocol (Wagg *et al.* 2014) and experimental microcosms (EcoTubes) to create and maintain a soil biodiversity and community composition gradient ranging from a highly diverse and complex soil community including soil organisms with a body size of 5000 μm or less, down to a low diversity simplified soil (sterilized soil). Excluding organisms based on body size parallels the physical simplification of soil functional guilds that can result from land management practices, such as tilling in agricultural systems (Brussaard 1997). Such a organismal size-based filtering allows for more accurate conclusions to be drawn about the possible effects that can come from these practices that physically disturb the soil inhabitants (Jansa *et al.* 2003; Postma-Blaauw *et al.* 2010; Köhl, Oehl & van der Heijden 2014; Säle *et al.* 2015). We maintained these microcosms for over a one-year period and monitored changes in fungal and bacterial community compositions as well as the productivity and community composition of plants, carbon sequestration and litter decomposition every two and a half months.

Materials & Methods

Experiment housing and setup

Miniature self-contained cylindrical growth chambers (EcoTubes) (23cm diameter x 34 cm height) were used as the experimental housing unit for this work as they provide a controlled and isolated environment where incoming pressured air and water are passed through hydrophobic (0.2 μm pore size) and hydrophilic (0.22 μm pore size) filters (all Millex®-FG_{50}; Millipore Corporation, Billerica, USA). This design minimizes greenhouse-borne microbial contamination, allowing the effects of manipulated microbial communities within each EcoTube to be more effectively assessed. A detailed description of these EcoTubes can be found in van der Heijden *et al.* (2015) (see photo in Appendix S1). To better ensure the sterility of the microcosm environments, all EcoTube components were sterilized by autoclaving at 120 °C for a minimum of 20 minutes, with the exception of the Plexiglas tops and the polyvinyl chloride bottoms. Since these materials deformed when autoclaved, they were sterilized by a 20 minute submersion in 0.5% hypochlorite followed by a 70% Ethanol with Tween 20 bath before being immediately placed in the laminar-flow hood to air dry. The soil and plant communities were also planted under these same sterile conditions in an effort to minimize contamination.

Soil substrate and inocula

The bottom of each EcoTube was filled first with a 1 cm layer of quartz stones (approximately 1 cm diameter) before being covered with a propyltex screen (0.5 mm mesh size Sefar AG, Heiden, Switzerland) to better accommodate drainage of excess water. As a sterilized substrate 5.5 kg (dry mass) of a 50/50 field soil/quartz sand mix sieved through a 5 mm mesh and autoclaved (120 °C for 90 minutes) was added on top of the screens in each EcoTube. All soil for this substrate came from a natural grassland near the Agroscope

Reckenholz research station in Zürich, Switzerland (47° 25’ 38.71’’ N, 8° 31’ 3.91’’ E). It was inoculated with soil collected from three agricultural fields with similar soil structures, but different management histories.

We used soils from three different management practices; “organic”, “conventional” and “intensive” to inoculate the microcosms with various soil communities. These three soil communities were used to better generalize our results across a gradient of site-specific land-use histories and characteristics. The three soil communities reflect a gradient from less intensive (“organic”) to intermediate (“conventional”) to intensive (“intensive”) agricultural management providing the opportunity to test whether there are indications that land-use intensity affects the ability of soil communities to provide ecosystem services. The first two soils came from the so-called DOK experimental field site in Therwil, Switzerland (47° 30' 8.9964” N, 7° 32' 21.8292” E). The DOK experiment was designed to assess different agricultural management practices, such as various fertilizer practices, on various ecological and agricultural characteristics of plots (see Mäder et al. 2002 for details). For the present study, soil was collected from four plots where the management practice was the addition of organic fertilizer (Site A, organic) and from another four plots where the management practice was addition of mineral fertilizer (Site B, conventional). The third soil was sampled from a plot in Freiburg, Germany (47° 58' 26.058” N, 7° 46' 31.5336” E) that had been continuously planted with the same crop (maize) for over 10 years (Site C, intensive). Details about soil characteristics of each of the three sites are provided in Appendix S2 in Supporting Information.

At all three sites soil was collected using four transects, one meter apart per plot, coring soil every four meters. Soil cores were mixed per site and homogenized by sieving through a 5 mm sieve. A sample from each of the sites was taken for a substrate characteristics analysis and no significant differences were detected (see Appendix S2). 250 g

of fresh soil from each of the different sites was further processed by wet sieving through a series of decreasing mesh sizes using 1L dH_2O to create four levels of soil community inoculum treatments containing species with body sizes < 5000 µm ("full soil"), < 100 µm, < 25 µm, and a sterilized inocula (created by autoclaving for 90 min at 120 °C). An earlier study from Wagg et al. (2014) demonstrated that this methodology successfully manipulates soil biodiversity and soil community composition. Body size is known to be a useful functional trait because it is directly associated with the metabolic rates, population density, generation time and food size of different soil organisms and thus can be used to form functional groups (Bradford *et al.* 2002). Furthermore, several studies have shown that agricultural intensification selects for small-bodied functional groups (Postma-Blaauw *et al.* 2010). To reduce the differences that sieving out the larger soil particles (i.e. nutrients) could render, soil material not passing through the sieves was collected, autoclaved and added back into the inoculum and mixed well. These four sieving treatments were replicated five times for each of the three management fields, resulting in a total of 60 EcoTubes. A single inoculum was added to the sterilized substrate in each of the 60 EcoTubes and the entire soil contents were mixed well. The inocula addition amounted to only 5% of the EcoTube soil volume, as we were interested in isolating the effects coming from differences in the soil microbial community, not from nutrient differences among the source sites.

In order to later measure litter decomposition, two 0.5 mm propyltex mesh litterbags (6 cm x 6 cm) were each filled with 1 g of dried *Lolium multiflorum*, sterilized, and buried just below the surface of the soil substrate in each EcoTube. One litterbag was removed and replaced at each harvest and the other remained for the entire duration of the experiment and removed at the end.

Plant community

Each EcoTube was planted with 12 individuals of the grass *Lolium perenne* and 12 individuals of the nitrogen-fixing legume *Trifolium pratense*, along with two individuals each of *Achillea millefolium* (forb), *Festuca pratensis* (grass), *Lotus corniculatus* (legume), *Plantago lanceolata* (forb), and *Prunella vulgaris* (forb), for a total of 34 individual plants per EcoTube (Lauber, Wagner & Gygax 2012). These species commonly coexist in natural Swiss grasslands. Moreover, *T. pratense* and *L. perenne* are the two main species that commonly co-occur in managed grassland (e.g. permanent grassland or as fodder crops within crop rotations) in Switzerland. Additionally, *T. pratense* and *L. perenne* are model species belonging to different plant functional groups (legumes and grasses) and known to respond differently to soil biota: legumes depend heavily on associations with their soil biota for increased performance while grasses are less dependent on associations with soil biota (Klironomos 2003; Wagg *et al.* 2011a, 2014). We included the five other plant species in the experimental communities at a lesser abundance because they commonly occur in managed grass-clover fields, and they also allow for a better assessment of diversity responses of the plant community.

Seeds of each species were surface sterilized by immersion in 2.5 % hyposodium chlorate for five minutes, then rinsing thoroughly in distilled H_2O. Surface-sterilized seeds were then plated onto 1% Agar in Petri dishes to germinate. In order to ensure that the seedlings of all species were at the same stage of development when planted, the seed germination process was staggered so that each species exhibited the presence of cotyledon(s) or radicle when transplanted. Seedlings were planted into one of 34 evenly spaced and randomly selected positions in the inoculated substrate of each microcosm. These experimental communities were set up over eight days and the day on which each was set up was used as a blocking factor in the subsequent analysis of variance (ANOVA).

Once planted and sealed, all EcoTubes were placed into a greenhouse compartment where natural light was subsidized by 400-W high-pressure sodium lamps in order to maintain an environment of 16 h / 25 °C days and 8 h / 16 °C nights with a light level above 300 W/m2. Twice weekly, the EcoTubes were watered on an individual basis with dH_2O to maintain gravimetric soil moisture in the range of 10–20%. However, since the greenhouse conditions maintain a constant environment, which does not reflect those found in nature that allow for temporal variation among species through time, we induced a variation in the watering regime to simulate an extended period without rain. The variation in precipitation was applied to all of the experimental communities at the same time by withholding watering for 10 days beginning five and a half weeks before each harvest. The plant communities were grown under these conditions for a total of 55 weeks.

Data collection

Over the 55-week growing period plant individuals were cut at 5 cm above the soil surface every 11 weeks to monitor changes in the plant community. EcoTubes were harvested following the same schedule in which they were planted. Plant individuals were counted and separated by species, dried at 65 °C and the biomass weighed. For each harvest we calculated the total biomass (net primary productivity = NPP) and a Shannon diversity index of the plant community (the sum of the biomass proportion of a species times the log proportion).

At each harvest one of the *L. multiflorum* litter bags was removed, the space was filled with a new, sterilized, and labelled litter bag, and then the removed bag was washed clean of soil, dried at 65 °C, then the remaining litter biomass was removed and weighed. Six randomly-spaced soil cores of a 1.7 mm diameter and the full depth of the soil substrate

(approximately 20 g each) were taken during each harvest. Soil core samples were mixed well and divided into separate sterile containers and frozen at -20 ° C for later analysis.

At 36 and 18 hours before being harvested each EcoTube was injected with 40 ml of ^{13}C labelled CO_2 (99 %) gas and sealed for one and two hours respectively. This was done to get a measure of the atmospheric carbon fixation and belowground storage efficiency ("soil C sequestration") of the soil communities in each EcoTube over time. Soils samples to be analyzed for ^{13}C content were frozen and lyophilized following methodologies recommended by Krab *et al.*(2012).

Molecular assessment of soil communities

Following each harvest, DNA was extracted from 500 mg of the homogenized soil samples taken from each EcoTube mentioned above. Extraction was done using the FastDNA® SPIN Kits for Soil (MP Biomedicals, Switzerland). Using a Quant-iT™ PicoGreen® (Molecular Probes, Eugene, OR) on a luminescence spectrometer (Perkin Elmer, LS 30, Rotkreuz Switzerland) the extracted DNA was quantified. All samples were then diluted to 10 ng / µl and used as DNA template in PCR reactions using the primers bRISArev and bRISAfor (FAM-labelled), targeting the 16s rDNA region with the cycling conditions and reagent concentrations outlined in Hartmann *et al.* (2005) and Wagg *et al.* (2014) for amplifying the bacterial community (see Hartmann *et al.* 2005 for full primer sequences and PCR cycling protocol). Fungal DNA was amplified using primers fRISArev and fRISAfor (FAM-labelled) targeting the region ITS1-5.8S-ITS2 following the reagent concentration and PCR cycling conditions outlined in Schneider *et al.* (2010).

Bacterial and fungal operational taxonomic units (OTUs) were determined by ribosomal intergeneric spacer analysis (RISA). This was carried out by mixing two µl of PCR product with 12 µl HiDi-Formamid and 0.2 µl MapMarker® 1000 (BioVentures,

Murfreesboro, TN) and subject to fragment analysis in an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Run conditions were set to an injection time of 30s at 1.5 kV and 10 s with a run time of 3000 s at 10kV. GeneMarker 1.91 genotyping software (SoftGenetics LLC, State College, PA) was used to characterize the unambiguous peaks of the amplified DNA fragments and the relative migration units were used as operational taxonomic units (OTUs). Peak intensities over the threshold value of 200 units and between 193 and 800 base pairs for fungi and > 800 florescence intensity with 150-600 base pairs for bacteria were scored as relative florescence units. The number of peaks (OTUs) that were determined by RISA were used as a measure of fungal and bacterial richness.

Data analysis

We used linear mixed-effects models to test the effect of soil diversity (by sieve size 5000µm, 100µm, 25 µm, and sterile) on the temporal stability of four ecosystem functions, net primary production (NPP), plant diversity (*H'),* litter decomposition, and soil carbon sequestration. The field from where the soil was collected (site A, B and C), and the planting/harvesting block were added as random terms. Temporal stability was calculated using the inverse coefficient of variation determined by μ/σ, where μ is the temporal mean and σ is the temporal standard deviation (Loreau & de Mazancourt 2008, 2013; de Mazancourt *et al.* 2013). Standard deviation is the square root of the variance so these terms are referred to interchangeably throughout as they are directly related. Furthermore, as previous research has illustrated that different plant functional groups associate differently with fungi and bacteria (De Deyn, Raaijmakers & Van der Putten 2004), the biomass of each plant functional group was also analyzed separately as additional measures of ecosystem functioning. All statistical analyses were completed using R software (version 3.0.0; The R

Foundation for Statistical Computing 2013), including the packages 'vegan', and 'nlme'. In all analyses significance was determined as a type I error of $\alpha < 5$ %. It is known that there are weaknesses in assessing solely community stability because of the way that fluctuations in the temporal mean and temporal variation (as measured by the standard deviation) in the stability calculation (μ/σ) can change the outcome and conclusions. For example, Gross et al (2014) pointed out that, contrary to previous beliefs about stability calculations, increases in stability do not necessarily mean that there is decreased variance in the system because substantial increases in the temporal mean values can hide a large amount of temporal variance that may exist. This is due to the inherent structure of this equation where, stability could be increased by the mean increasing relative to the standard deviation, but also by the standard deviation decreasing relative to the mean. This means that simply looking at the stability values alone could yield experiment conclusions that would be relatively impractical for application as management efforts targeting the maximization of mean performance and the minimization of temporal variability; such as would be desired for agricultural systems. Therefore, in an effort to account for these inherent misinterpretations, we present the temporal means and standard deviations of the performance of the ecosystem functions throughout.

Assessment of soil biodiversity and temporal asynchrony

Soil fungal and bacterial OTU communities were assessed using mixed effect models as described for the analysis of individual ecosystem functions. In order to detect potential species asynchrony in the fungal and bacterial communities we first assessed the correlation between each fungal and bacterial OTU with each ecosystem function within a given harvest (i.e. at a single time point). OTUs that were significantly ($p < 0.05$) and positively correlated with an ecosystem function within any given harvest were considered and their covariance

through time for each EcoTube was calculated. We then summed the negative covariances among OTUs to quantify the level at which different OTUs performed differently at different times (Schluter 1984). The sum of negative covariances quantifies potential asynchronous interactions among soil species. Asynchrony (*sensu* Loreau & de Mazancourt 2013) could not be calculated since the value of an OTU in our data is a relative measure and summing the OTUs (as one would do to calculate asynchrony) would always equal 1; i.e. since the OTU abundances are relative proportions and the variation in their summed total is always 1 and thus the community level variance over time is therefore always 0. Moreover, the sum of the relative abundance of OTUs associated with an ecosystem function cannot be reliably linked to the observed valued of an ecosystem function as each value is sample dependent. Therefore we opt for the sum of negative co-variances among OTUs that might be associated with an ecosystem function at any given time. We are aware that there are flaws in using covariation to come to conclusions about the stability of ecosystem functioning, as pointed out by Loreau & de Mazancourt (2008) and Carnus et al. (2015), where using this measure when comparing more than one species can lead to species' effects canceling each other out. However, this was the best readily available methodology for beginning to pry into the mechanisms underlying how the changes in the soil community correspond with fluctuations and stability in the performance of multiple ecosystem functions with the data set that was available.

We used Pearson correlation to assess associations between the fungal and bacterial community asynchrony and the stability of each ecosystem function over time. Additionally, correlation coefficients were calculated using the sum of negative covariances containing all OTUs (not just those associated with each ecosystem function) to assess whether associations were generated by chance alone.

Results

Sieving effects on soil microbial diversity

Fungal richness generally declined with decreasing sieve size, demonstrating the efficiency of the soil sieving treatments in creating a gradient of soil diversity ($F_{3, 48,}$ $P < 0.0001$, Fig. 1A). The fungal community was most rich in full soil community treatment (5000 μm) until the 33rd week when the differences among the soil community treatments became less defined. The differences among the soil sieving treatments in bacterial richness was variable from harvest to harvest but overall was different among the treatments ($F_{3, 49,}$ $P < 0.0001$), and increased over time (Fig 1B).

Effects of soil biodiversity on ecosystem stability

Loss of soil diversity negatively affected the stability of the four ecosystem functions measured in this experiment (Table 1; Fig. 2). Temporal stability was lowest in the sterilized soil community treatment for each of NPP, plant diversity, litter decomposition and C sequestration. This was due to a reduction in the temporal mean as well as to an increase in the temporal variance compared with the full soil treatment.

Soil biodiversity, temporal asynchrony and ecosystem functioning stability

Increases in fungal community OTU asynchrony (for those fungal taxa associated with each function) in the EcoTubes was positively correlated with higher stability in overall NPP ($r = 0.26$, $p = 0.057$), legume biomass ($r = 0.52$, $p < 0.001$), forb biomass ($r = 0.34$, $p = 0.013$), and plant diversity ($r = 0.43$, $p = 0.001$; Table 2; Fig. 4A). Increasing bacterial community asynchrony and was significantly and positively associated with the stability of

grass biomass production ($r = 0.26$, $p = 0.051$), and decomposition ($r = 0.33$, $p = 0.015$; Table 2; Fig.4B). Furthermore, when correlation coefficients were calculated using the sum of negative covariances containing all OTUs (not just those associated with each ecosystem function) for both fungal and bacterial taxa, all relationships were not significant (dashed grey lines in Fig. 4A–B). This suggests that our methodology of isolating only associated OTUs for analysis does pick up a signal in the temporal soil microbial community dynamics that may be underlying the temporal performance of the ecosystem functions assessed.

An additional analysis testing the structure of negative covariance of fungal OTUs over time, our measure of soil fungal species asynchrony, across our sieving treatment showed that asynchrony did decrease with decreasing sieve size (Table 3; Appendix S5A) for the fungal OTUs associated with NPP, plant diversity, and C sequestration ($r = 0.57$, $p < 0.001$; $r = 0.61$, $p < 0.001$; and $r = 0.5$, $p < 0.001$ respectively). Using this same method of analysis looking at the covariation of the abundance of soil bacteria OTUs over time across the sieving treatments, the only obvious correlation was a positive trend in asynchrony of soil bacterial species associated with the grass biomass production with decreasing sieve size ($r = 0.31$, $p < 0.05$, Table 3; Appendix S5B).

Discussion

It has been observed that biodiversity is a key underlying mechanism behind maintaining the performance of ecosystem functions through time (Hooper *et al.* 2005; Loreau & de Mazancourt 2013). Here we found the reduction of soil biodiversity and the simplification of the community composition led to a greater destabilization in the four ecosystem functions analyzed. This corroborates with our expectation and hypothesis that soil biodiversity is vital for maintaining the stability of multiple ecosystem functions. By experimentally simplifying the soil community composition based on body size, we reduced

the detectable soil fungal and bacterial communities. Consequently, our general decline in soil diversity and simplification of community composition can be associated, not only with the decline in multiple ecosystem functions, but also the reduction in their temporal stability emphasizes the importance of the maintenance of the belowground soil biodiversity and community composition for sustaining various ecosystem processes over time.

Effects on plant communities

The NPP response over time in our system followed a similar pattern among the different sieving treatments, except in those containing the sterilized soil inoculum. The NPP in EcoTubes with the sterilized soil community addition was lowest at the first harvest and then increased to its highest level at 22 weeks (Fig. 2A). However, from there forth, NPP continuously plunged downward until the end of the experiment. This single boom at one harvest but lower production levels at all other time points yielded the lowest temporal mean biomass production among all sieves. Looking at the temporal mean and temporal standard deviation highlights how the resulting pattern of the stability among the sieving treatments were driven more so by the differences in the temporal mean rather than the differences in the standard deviation, which were smaller among treatments. Furthermore, we can also see that the considerably lower stability of the sterilized soil arises from the increased power that the standard deviation of this treatment had on its lower mean value in the stability equation. These results are a nice example to further highlight the previous work pointing out that variations in overall stability can be driven by differences in the interplay of the temporal mean and temporal standard deviation within the stability calculation, and therefore, it is best to analyze them separately to get a true picture of what is going on the system (Gross *et al.* 2014; Hautier *et al.* 2015).

Digging into the mechanisms driving the differences among the sieving treatments by separating out the NPP by plant species and their according plant functional groups we observed vast differences in the structure and temporal fluctuations of the proportions of plant species making up the biomass under each sieving treatment (Figure 3). We saw that the NPP in the sterilized soil inocula was heavily driven by the changes in grass biomass over time, as it made up the greatest proportion of the biomass. Furthermore, the dominance of grasses in our system where soil communities were reduced the most (sterile inocula), coupled with the near absence of the legume and forb plant species, explains why the sterilized soil communities had by far the lowest stability in overall NPP (Appendix S3–S4). These results are similar to what was found in previous work linking soil biodiversity and differences in plant functional group productivity (Bradford *et al.* 2002; Wagg *et al.* 2014). Higher legume biomass production over time in the two most diverse soil treatments matched that of previous studies linking increased legume biomass to the presence of AMF (Pellkofer *et al.*in process; Wagg *et al.* 2014; van der Heijden *et al.* 2015). This would logically fit with the size delineation of sieves in this experiment, as the average size of an AMF spore is around 40 μm (Marleau *et al.* 2011), meaning that they would only be present in the two most diverse sieving treatments. The presence of these plant-benefiting fungal partners allows for legumes to optimally function by obtaining more of the available nutrients to compete against robust grass species (van der Heijden *et al.* 1998; Marler, Zabinski & Callaway 1999; Maherali & Klironomos 2007; Wagg *et al.* 2011b; a).

Plant diversity was highest in the most diverse soil treatments (5000 and 100 μm) and it showed an ever-increasing pattern over the entire duration of the experiment. However, the diversity of plants in the sterilized soil treatment hovered at just above zero throughout the full year (Fig. 2B). The temporal mean of plant diversity was by far the lowest in the sterilized soil treatment and increased with increasing soil biodiversity. The sterilized soil

also had the highest variance in its plant diversity over time, which compiled with the low trend in the mean value to yield the lowest level of stability in plant diversity as well. Moreover, with greater plant diversity over time, the higher diversity soil treatments exhibited an interplay of plant biomass fluctuations among the three plant functional groups. A compositional interaction where drops in the biomass of one functional group were compensated for by booms in the production of other groups simultaneously, resulting in greater overall community stability. These asynchronous fluctuations among plant functional groups over time matches what we found in our previous work showing that the presence of a soil community allow for more asynchronous plant species fluctuations and ultimately more stable plant community productivity over time (Pellkofer *et al.* in process). This structure of interchange in the diversity of the plant community within the higher soil diversity treatments could be a direct or indirect result of the interactions of soil mutualists and antagonists (Putten, Dijk & Peters 1993; Bezemer *et al.* 2010; Wagg *et al.* 2014).

Effects on the stability of decomposition and carbon storage

The presence of the least disturbed, or rather most complete and diverse soil community, resulted in the greatest decomposition of litter in the first harvest and maintained a level of decomposition across all harvests that was more stable over time than the other soil sieving treatments (Table 1; Fig. 2C). The presence of more microbial soil community members would explain the greater decomposition levels over time and the overall greater stability of decomposition in the full soil treatment as compared to the less diverse soil treatments, as it is now well known that the efficiency of organic matter decomposition is directly linked to the abundance and functional diversity of the soil microbial community (Heemsbergen *et al.* 2004; Bonkowski & Roy 2005). The amount of litter decomposed in EcoTubes with the other three less diverse soil sieving treatment inocula followed a similar

pattern of being low in the first harvest, higher at the second harvest then diving off in the third harvest before ever increasing until the final harvest. The amount of litter decomposed in the sterilized soil treatment was consistently lower than all other treatments at every harvest time point yielding a significantly lower temporal mean as compared to the other more diverse treatments. The power of this low mean along with a high temporal variance translated over to lower stability as well.

The patterns we found in the amount of soil C sequestered in our systems could be best explained by the community composition and characteristics of the plant community, based on the work that has shown the direct link between functional plant group abundances and the efficiency of photosynthesis (Fornara & Tilman 2008; De Deyn *et al.* 2011). And in fact additional correlation tests revealed a positive link between standardized soil C sequestration values and plant diversity ($r = 0.22$, $p < 0.001$).

Soil microbial community structure over time and ecosystem functioning stability

The insurance hypothesis implies that a greater diversity of taxa provide insurance through the greater probability that some species will be able to maintain ecosystem functioning at any given time (Naeem & Li 1997; Yachi & Loreau 1999). Consequently, the stability of functioning at the community level is insured through the asynchronous fluctuations in the soil taxa over time. Here we found that the greater negative covariance among microbial taxa that could be associated with an ecosystem function may indicate the presence of asynchrony. Furthermore, the sum of negative covariances among soil microbes that can be associated with an ecosystem function declined with soil sieving, further indicating a potential insurance effect of a more complete soil community composition.

Specifically, our results showing a positive correlation between increased fungal OTU asynchrony and higher stability in NPP, legume and forb biomass and plant diversity hint at the importance of fungal community temporal dynamics for maintaining the stability of the plant community within an ecosystem. The fact that the ecosystem functions that showed the strongest links with fungal species asynchrony were plant responses highlights the intimacy of the partnership that is formed between fungi and plants. Such a result exemplifies the complexity of interactions and feedbacks that soil and plant community diversity and composition can have on each other simultaneously.

The positive correlation between the asynchrony of the soil bacterial community and decomposition highlights the important role that temporal changes in the soil bacterial community play in breaking down soil organic litter. This could be because it allows for a larger portion of the functional niche space in the decomposition process to be occupied by different microbes, thus, increasing the overall efficiency of the function. The positive link between increasing bacterial species asynchrony and the stability of grass biomass production is likely tied to the inherent condition of systems with lower soil biodiversity tending to be both bacterially dominated and having high levels of grass biomass production. This can occur because grasses tend to be better competitors for soil nutrients, such as N, when nutrient availability is low. This was perhaps the case in our system since grasses often dominate plant communities when soil biodiversity is low and the soil organisms that supply nutrients and assist in plant nutrient uptake are absent from the system (van der Heijden 2003). While soil ecosystems that are largely dominated by bacterial communities are also likely to occur in our system as larger organisms are filtered out because bacterial populations can explode when there is a lack of predators as well as few competitors within the same niche space (Olsen & Bakken 1987; Kang & Mills 2004; Wertz *et al.* 2006). Nonetheless, the absence of obvious connections between the fluctuations of the soil bacterial

community over time and the stability of all other measured ecosystem functions points out the likely weaknesses in the methodologies utilized in this study for separating out the effects of specific bacteria or groups of bacteria in soil biodiversity and ecosystem functioning stability relationships.

Although summing the negative covariance can have some flaws as a mathematical tool for analyzing community change in systems when there are more than two species present, it does appear to be a good start for getting at some of the mechanisms underlying the link between differences in stability originating from the differences in the soil communities induced by the sieving treatments. Such results provide provides the initial steps into prying into the functional importance of the temporal changes in the soil microbial community.

Outlook

Although this work elucidates some of the links between changes in soil community biodiversity and the stability of multiple ecosystem functions over time, it simultaneously introduces more specific ideas of the interactions that should be further investigated in order to really understand the mechanisms underlying the relationship. Based on the results of this study, we suggest that more work be done to focus more explicitly on the bacterial communities to better understand the fluctuations of specific species over time and across soil diversity treatments. We suggest using more refined molecular methods to precisely identify species of bacterial, and also fungi, and track their population dynamics within a controlled system over time. Tying specific groups of organisms and critical thresholds of soil biodiversity to the temporal stability of ecosystem functioning could prove useful as a template off which future management efforts to maintained soil biodiversity that stabilizes

ecosystem functioning could be based. Furthermore, having data on the fluctuations of specific soil organisms and specific functional groups over time would allow for more mechanistic links to be drawn between changes in temporal soil community dynamics and the stability of ecosystem functioning. Moreover, it will be critical to investigate the differences in soil community composition that arise from various land management practices to observe how anthropogenically induced soil biodiversity losses and shifts in community composition link to changes in the stability of multiple ecosystem functions.

The clear links that we found between increasing fungal and bacterial species asynchrony and increased stability in the functioning of plant communities over time highlights the promise that exists for manipulating and maintaining soil microbial community diversity as a tool for stabilizing plant systems. The knowledge of how to better manage soils biodiversity in a way that encourages greater ecosystem stability will become increasingly valuable as we face the unknown future challenges that may arise from a rapidly changing climate.

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Tables

Table 1. ANOVA results for the effects soil sieving treatment (Sieve) on the temporal mean, the temporal standard deviation (SD) and the stability of the performance of the ecosystem functions NPP, plant diversity, decomposition, C sequestration. Results showing the effects on the biomass of the three plant functional groups separately are shown in the supplemental material. The temporal trends of these different ecosystem functions are shown in Figure 2.

		NPP		Plant diversity		Decomposition		C sequestration	
	Df_{num}	denDF	F	denDF	F	denDF	F	denDF	F
Mean	3	47.4	6.78***	49.0	120***	49.1	3.48*	51.1	3.13*
SD	3	294	4.45**	294	108***	284	7.99***	294	14.1***
Stability	3	50.5	12.7***	49.3	3.95*	47.1	2.07	30.1	2.33·

Df_{num} = numerator degrees of freedom; denDF = denominator degrees of freedom; F = F-ratio; · = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Table 2. Correlation coefficients (r) and the significance of correlations (p) between the soil fungal and bacterial OTU covariation (pseudo asynchrony) associated with the stability of each ecosystem function. Significant results are shown in bold text and those with $p < 0.06$ are italicized to highlight their importance. See full results plotted in Figure 4.

	Fungi		Bacteria	
	r	p	r	p
NPP	*0.26*	*0.057*	0.07	0.587
Plant diversity	**0.43**	**0.001**	0.13	0.328
Decomposition	0.19	0.183	**0.33**	**0.015**
C sequestration	0.15	0.295	0.11	0.423
Legumes	**0.52**	**<0.001**	0.11	0.418
Grasses	**0.34**	**0.013**	*0.26*	*0.051*
Forbs	**0.28**	**0.046**	0.07	0.595

Table 3. Correlation coefficients (r) and the significance of correlations (p) between our decreasing soil sieving treatment gradient (5000 μm down to sterilized soil) and the covariation of the key soil fungal and bacterial OTUs (pseudo asynchrony) for each ecosystem function (with the 3 functional group. Additionally the control correlation analysis of sieve with all OTUs (not just those associated with each function) is also included in italicized text. Significant results are shown in bold text. See full results plotted in appendix S5A-B.

	Fungi		Bacteria	
	r	p	r	p
NPP OTUs	**0.57**	**< 0.001**	0.07	0.58
Plant diversity OTUs	**0.61**	**< 0.001**	0.12	0.39
Decomposition OTUs	0.17	0.23	0.05	0.70
C sequestration OTUs	**0.50**	**< 0.001**	0.15	0.26
Legume biomass OTUs	0.74	< 0.001	0.10	0.48
Grass biomass OTUs	0.24	0.08	**0.31**	**<0.05**
Forb biomass OTUs	0.52	< 0.001	0.18	0.18
All OTUs	*0.08*	*0.56*	*0.06*	*0.65*

Figures

Fig. 1. Mean A) fungal and B) bacterial OTU richness +/- SEM over time (weeks) across the sieving treatments with letters above indicating significance among the sieving treatments within each harvest time point.

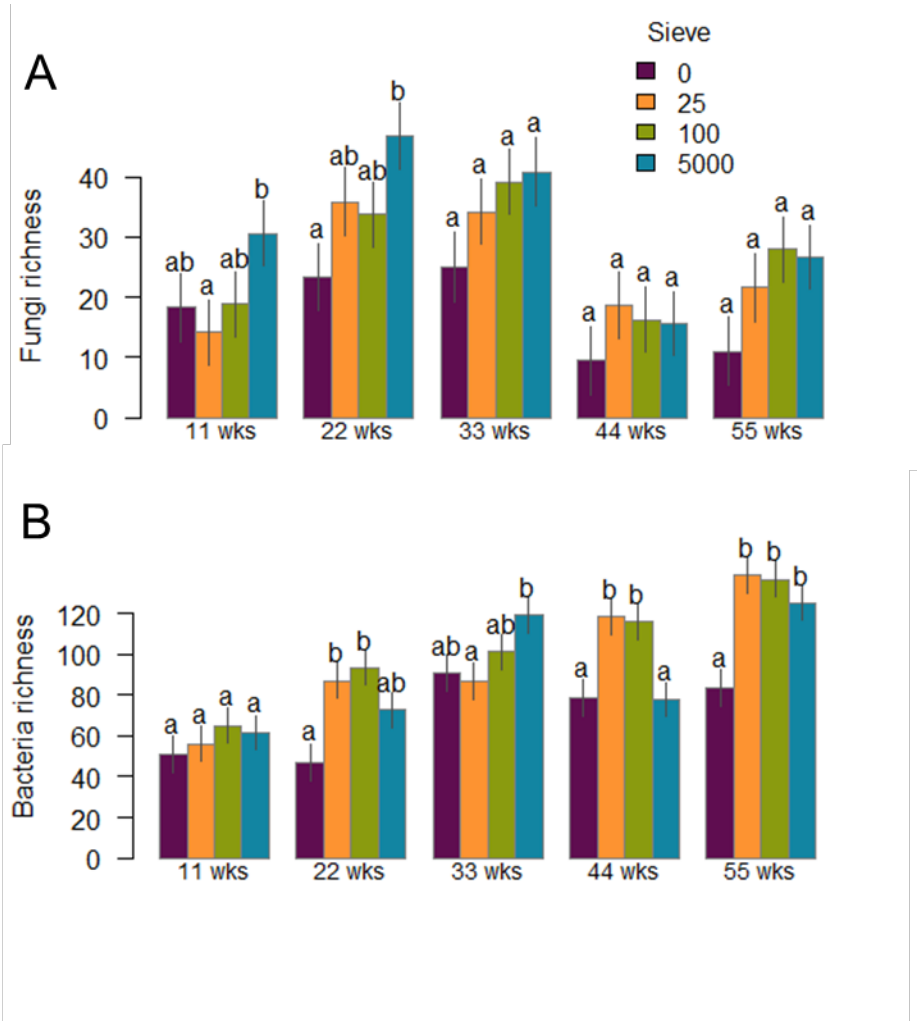


Fig. 2. Mean performance +/- SEM of the four measured ecosystem functions over time for each sieving treatment, along with the associated temporal means +/- SEM, temporal standard deviation +/- SEM, and overall stability +/- SEM for each sieving treatment.

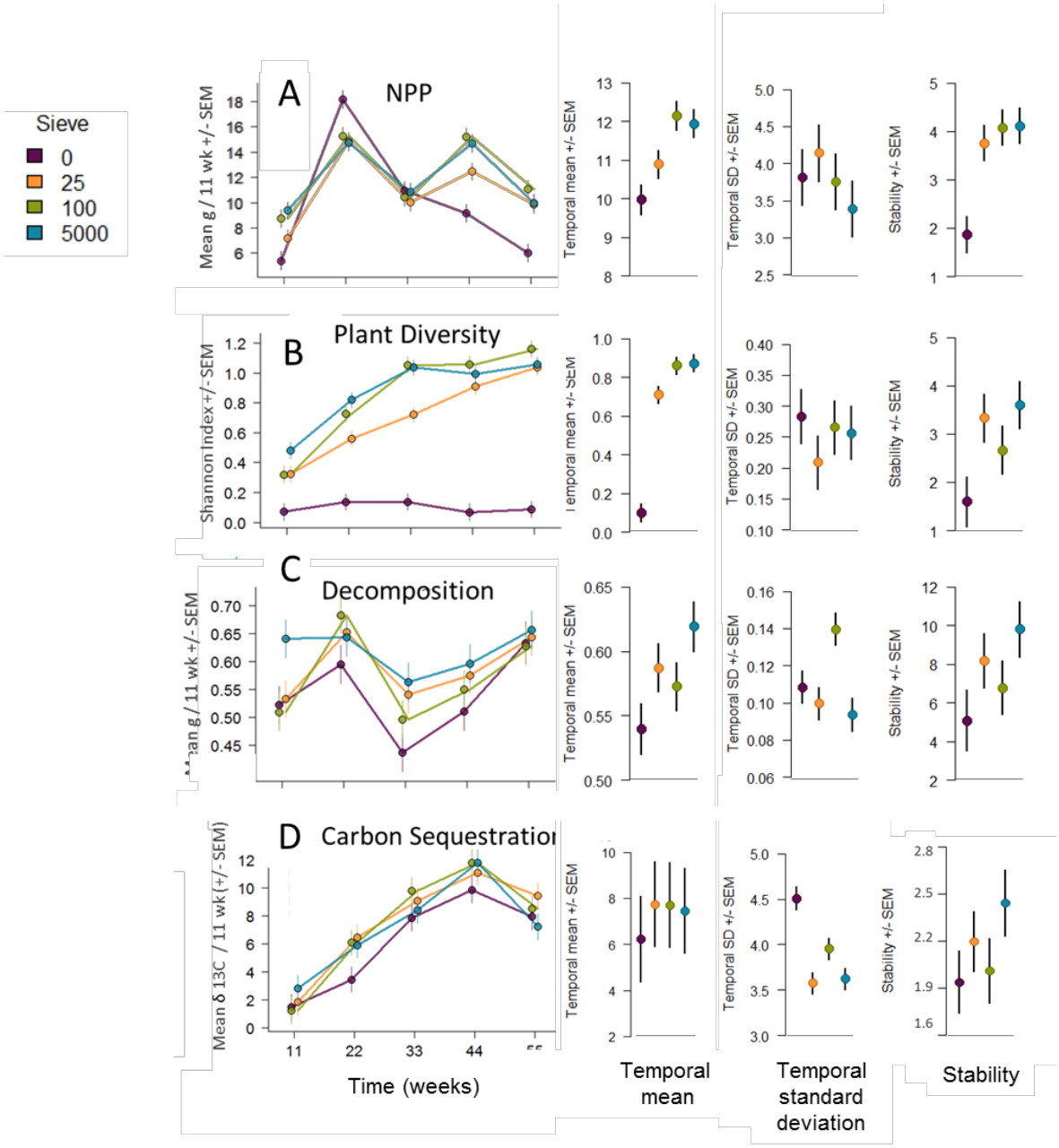


Fig. 3. Initial plant species proportions along with changes in plant species proportions over time under each soil sieving treatment with decreasing soil sieving treatments in blocks of decreasing soil biodiversity (5000 µm - sterilized) from left to right.

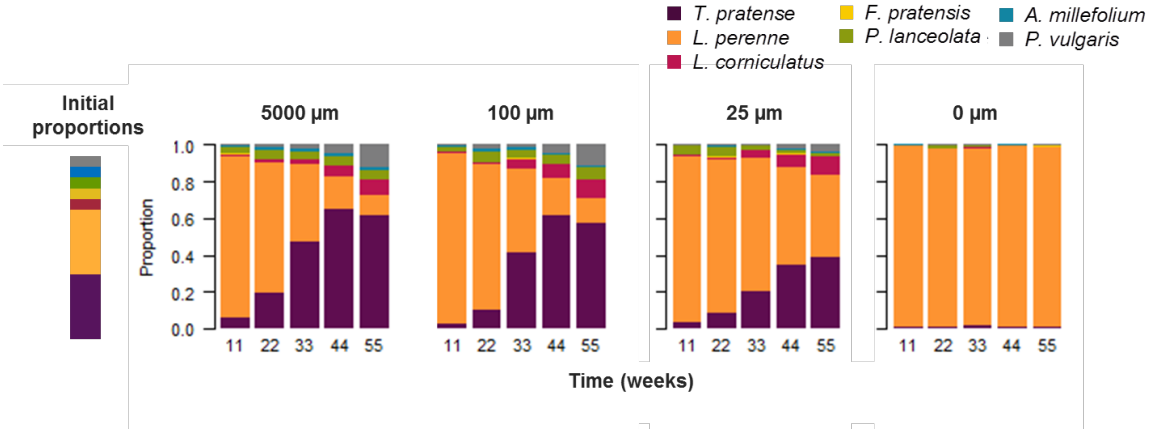
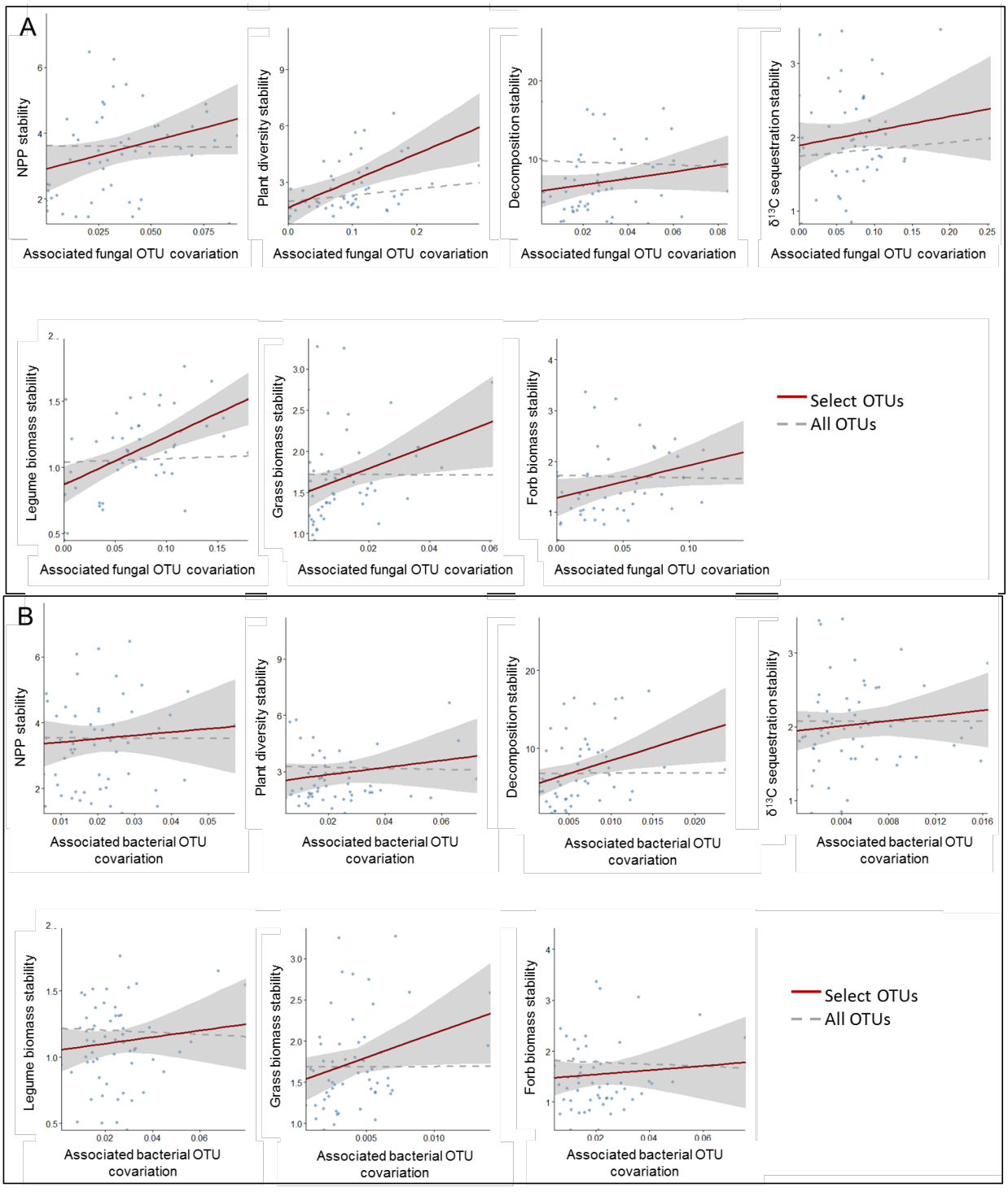
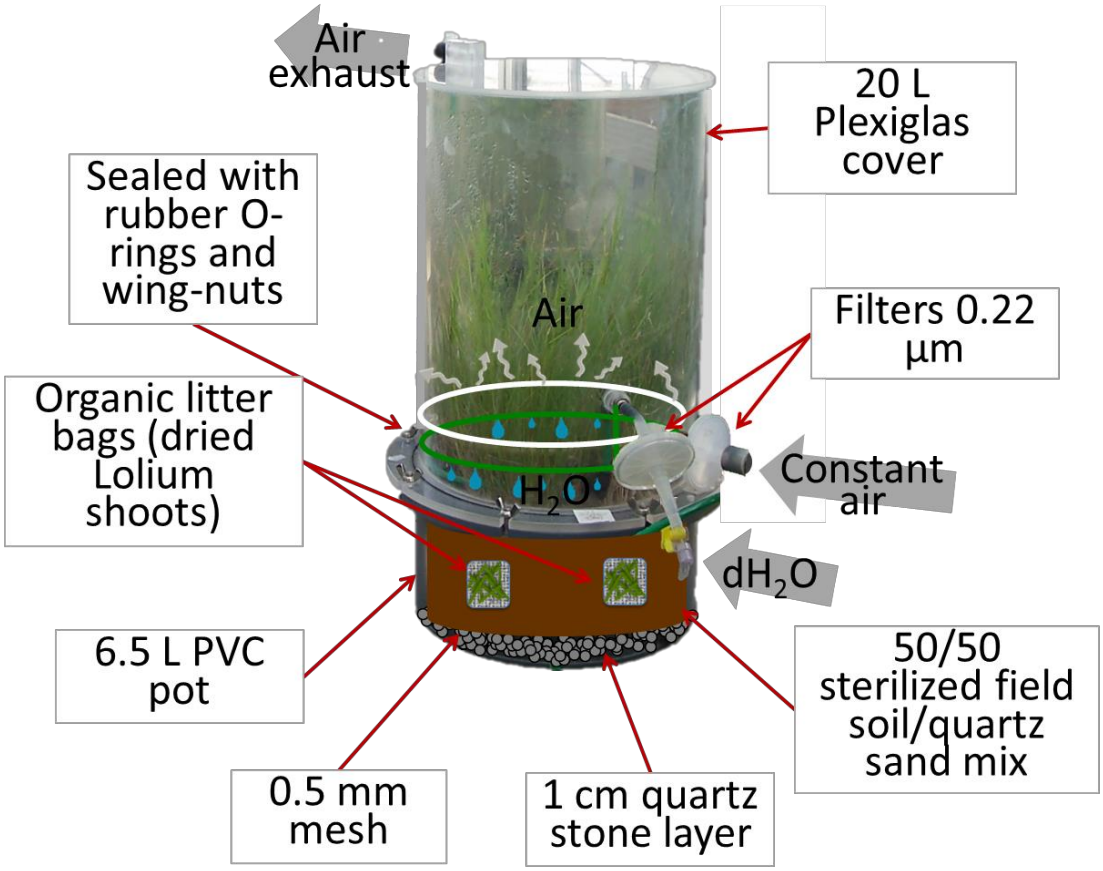


Fig. 4. Correlation coefficients between the soil A) fungal and B) bacterial OTU covariation (pseudo asynchrony) associated with the stability of each ecosystem function on the x-axis and the stability of the function on the y-axis with regressions from linear models in red with spread of the SEMs, along with the dashed grey line showing the analysis using all OTUs rather than just those associated with the ecosystem function. Ecosystem functions of NPP, plant diversity, decomposition, and C sequestration are shown respectively in the panels in the top row from left to right, with the additional analyses of the biomass of the legume, grass and forb biomass shown respectively in the bottom row from left to right.



Supporting information

Appendix S1. EcoTube microcosm design.



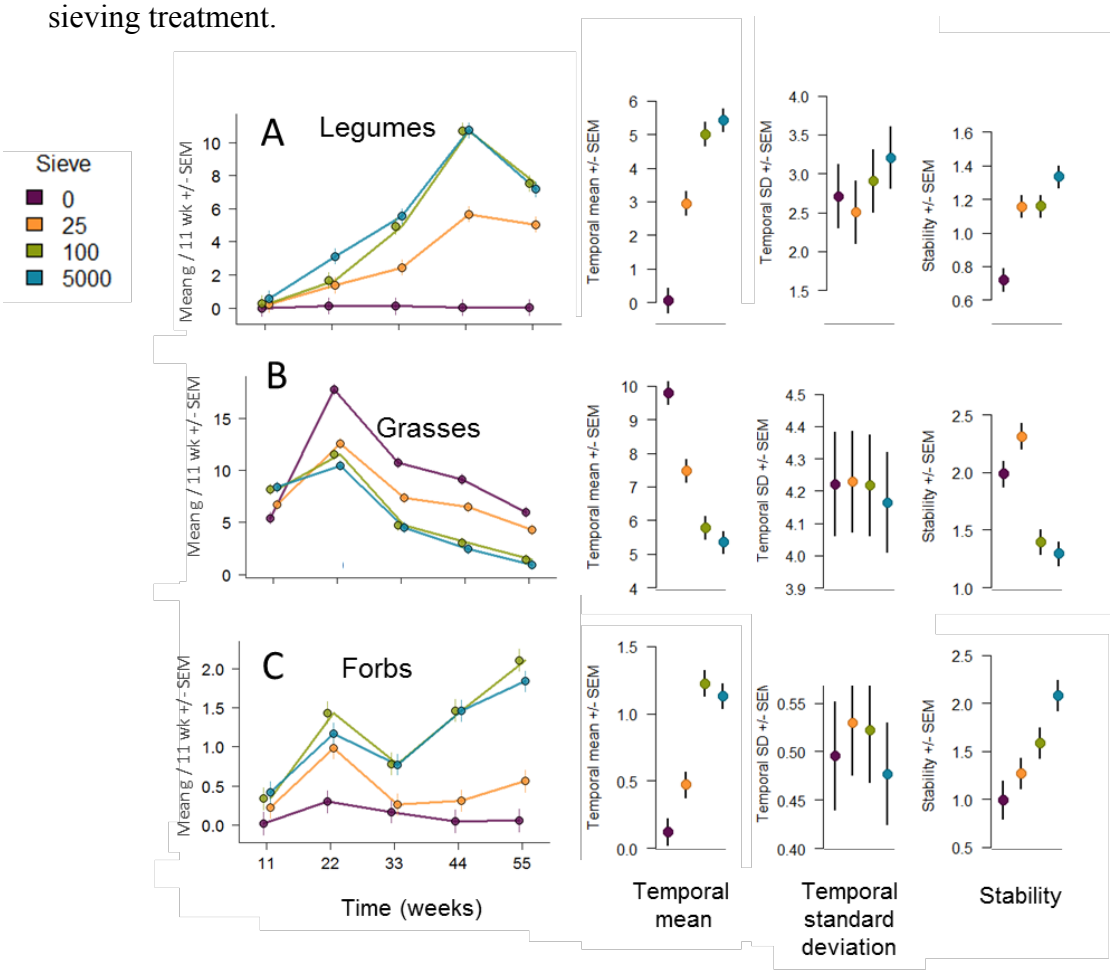
Appendix S2. Inocula soil history with initial soil properties analysis results. pH (pH) and $mg \cdot kg^{-1}$ of plant available potassium (K-Test) and magnesium (Mg-Test) content extracted with ammonium acid-extraction, CO_2 and $CaCl_2$. Ammonium acetate-EDTA (pH 4.65) was used to extract amounts of K, Mg, and Ca in $mg \cdot kg^{-1}$.

Term	Management	Site name & location	pH	K-Test*	Mg-Test*	K*	Mg*	Ca*
Site A, organic	organic fertilizer, "bio-Organic"	DOK† trial, Therwil, Switzerland	7.9	1.9	9.9	61.1	206	6228
Site B, conventional	mineral fertilizer, "Konventionell"	DOK trial, Therwil, Switzerland	7.4	2	10.4	59.7	202	6074
Site C, intensive	Intensive, 10-year maize mono cropping	Private farm, Freiburg, Germany	7.4	1.7	10.8	65	216	6533

* Results units are mg/kg

† Biologisch-dynamisch, organisch-biologisch and konventionell (DOK) trial. See www.fibl.org for more information about the study design (Mäder *et al.* 2002).

Appendix S3. Mean performance +/- SEM of the biomass production for the three plant functional groups over time for each sieving treatment, along with the associated temporal means +/- SEM, temporal standard deviation +/- SEM, and overall stability +/- SEM for each sieving treatment.

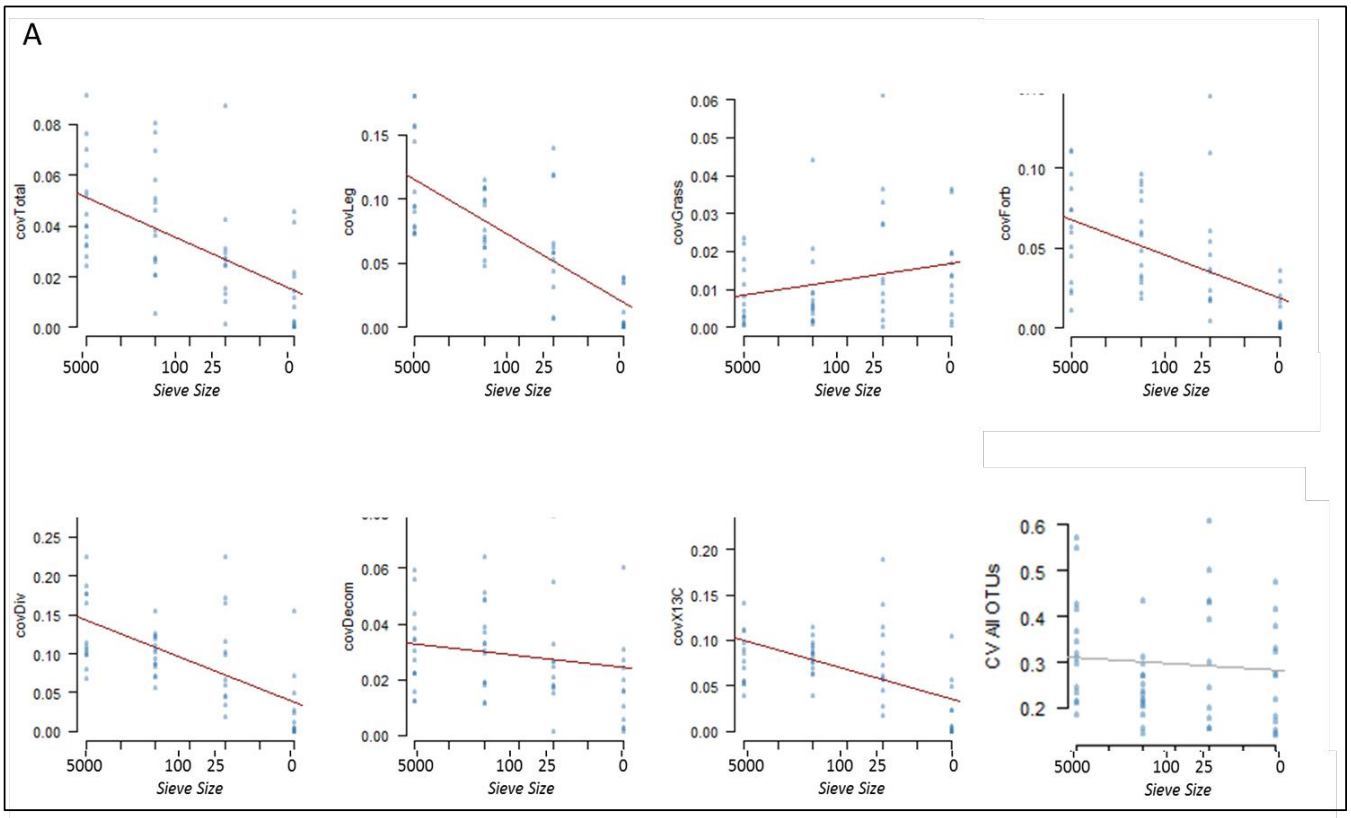


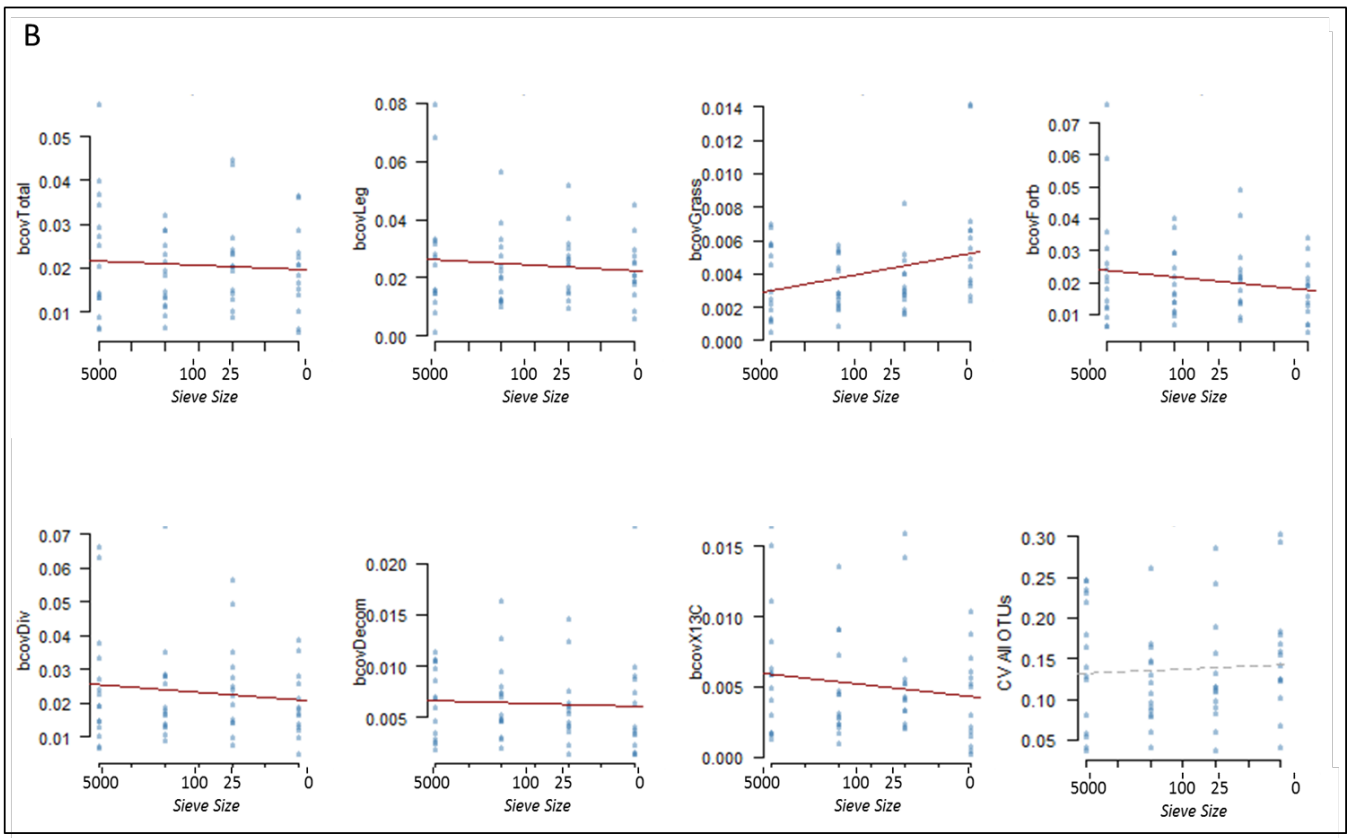
Appendix S4. ANOVA results for the effects soil sieving treatment (Sieve) on the temporal mean, the temporal standard deviation and the stability of the production of legume, grass and forb biomass.

		Legumes		Grasses		Forbs	
	Df_{num}	denDF	F	denDF	F	denDF	F
Mean	3	50.8	58.5***	48.0	51.1***	54.0	24.7***
SD	3	294	1.85	294	1.92	294	0.26
Stability	3	49.4	18.3***	50.5	22.3***	47.0	8.38***

Df_{num} = numerator degrees of freedom; denDF = denominator degrees of freedom; F = F-ratio; · = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Appendix S5. Correlation between the soil A) fungal and B) bacterial OTU covariation (asynchrony) associated with the stability of each ecosystem function and our decreasing soil sieving treatment gradient (5000 µm down to sterilized soil) along with the control comparing the covariation of all OTUs.





Discussion

"In the end, we will conserve only what we love;
we will love only what we understand;
and we will understand only what we are taught."
– Baba Dioum, Senegalese ecologist

General Discussion

We now understand, due to extensive work in the field of ecology, that our society both directly and indirectly relies upon the soil to provide us with the vital natural resources and ecosystem services that we utilize every day for our comfort and survival (Wardle *et al.* 2004; Hooper *et al.* 2005; Bardgett & Wardle 2010). The air we breathe, the food we eat, the material that we use to build shelter, and what we construct our shelters upon—they are all intricately linked to the soil. Furthermore, we now know that the levels of biodiversity that reside in the soils are the key determinate to how well these soils function (Wagg *et al.* 2014; Bradford *et al.* 2014). But as we are advancing the technologies that allow us to better peer into this black box of mystery that is the microscopic world that operates beneath our feet, we are beginning to pick apart the species and functional roles that different organisms in the soil fulfill, and as we do so, we are starting to realize just how much we do not know and understand about how these complex systems work and how the interactions among soil species determine the success of the provision of ecosystem services (Balvanera *et al.* 2006). We are now seeing how complicated the task of linking soil biodiversity and ecosystem functioning really is, due to the complex web of interactions that occur among soil species (competition, amensalism, mutualism, predation, parasitism, pathogenic, etc.) and how they operate in such a wide range of functional roles and guilds (root herbivores, decomposers, ecosystem engineers, mineralizers, immobilizer, nitrifiers, denitrifiers etc.) (Brussaard 1998, Wardle 2002, Paul 2007).

As we are facing serious losses of soil biodiversity on a global level as a result of anthropogenic activities (Helgason *et al.* 1998; Verbruggen *et al.* 2010; Tsiafouli *et al.* 2015), and the things that we are doing to provide for our human needs on an immediate short-term basis are incrementally knocking out certain species on different spatial and temporal scales, science is telling us that this this is an extremely dangerous game to be playing (Rockström *et*

al. 2009). Every action that degrades the soil sets ecosystem interactions in motion, the effects of which we cannot predict (Bardgett & Wardle 2010). As changes in the climate around us are increasing in frequency and intensity, with ever-growing anthropogenic pressures on the earth from an expanding human population, there has never been a more important time than now for ecological research to progress our knowledge and understanding of our impacts on the environment.

This dissertation strove to advance our knowledge within this realm of ecology, and introduced steps that evolve our understanding of just how important soil biodiversity is for the performance and stability of multiple ecosystem functions. By collecting soil from agricultural fields of varying land-management intensities and manipulating the contained soil biodiversity to make various inocula treatments, this work was able to directly demonstrate how the sole presence of soil biota was a significant and powerful determinant of the ability of plants to make biomass, the number of different plant species that the model systems were able to support, and finally, if those species were able to interact asynchronously to temporally stabilize the productivity of the plant community as a whole (Chapter 1; purple boxes in Fig 1). This nicely expands our understanding beyond previous studies showing that plant species diversity promotes primary productivity and stability in grassland ecosystems, as well as those demonstrating how soil community characteristics influence the productivity and composition of plant communities. We further elucidated how decreases in soil biodiversity along a gradient negatively affect the performance of multiple ecosystem functions, including plant productivity, plant diversity, decomposition, and soil nutrient uptake, retention and cycling, independent of the intensity of land-management practice from which the original soil inocula was taken. But interestingly, we found that when we combined all ecosystem-functioning measures into a single metric of ecosystem multifunctionality, the empirical and mathematical methods that are used to calculate that

single value determined whether differences in the performance of ecosystem multifunctionality were detectable or not among sites. This is an important point to note for future work as it demonstrates how the way that metrics of multifunctionality are developed affect not only the ultimate findings of the study, but also how comparable the results will be to future ecosystem multifunctionality work (Chapter 2; green boxes in Fig 1). Our findings also demonstrate that the negative effects that the loss of soil biodiversity have on multiple ecosystem functions found in previous work (Wagg *et al.* 2014) remain when the duration of the study period is doubled. This is important for considering the longer term implications that soil biodiversity loss might have on the functioning of the ecosystem. Furthermore, we discovered that soil biodiversity losses along a gradient negatively affect the temporal performance, increase the temporal standard deviation, and therefore decrease the stability of plant productivity, plant diversity and litter decomposition in a model system (Chapter 3; blue boxes in Fig 1). Moreover, our initial effort to pick apart the mechanism underlying these result by examining the specific temporal changes in soil community revealed that higher fungal taxa diversity increased stability in ecosystem functioning and fungal taxa asynchrony over time. This further demonstrates the complexities of soil community interactions and suggests a plausible and promising route forward in soil biodiversity–ecosystem functioning research.

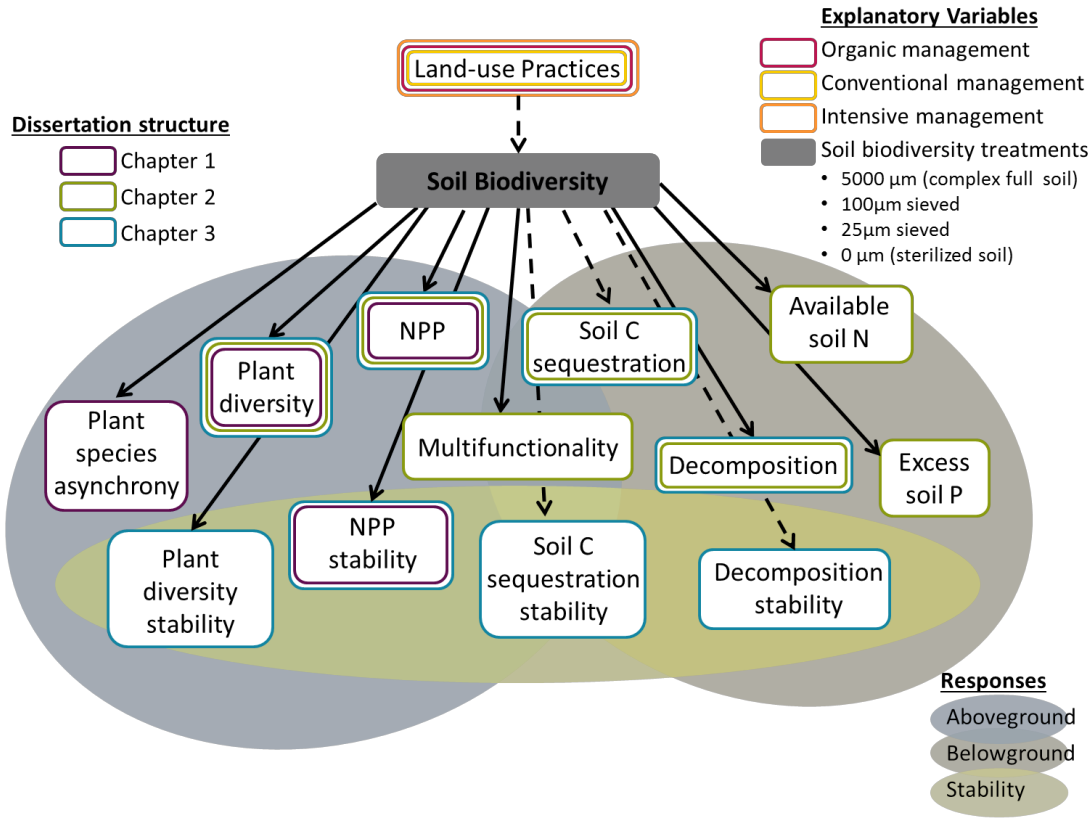


Fig 1. This diagram shows the significant positive effects that we found through this work in solid lines. It is important to note however, that this is extremely simplified and a more accurate depiction of the systems would have arrows going in both directions and interconnecting practically all of the response variables, as well as a whole host of other boxes on the sides with their own feedbacks in the system. A full diagram would be a very complex web, which highlights the difficulty of attributing cause and effect in natural ecological systems.

Most importantly, the complex nature of the results of this work highlight how the nuances underlying the soil biodiversity–ecosystem multifunctionality and stability relationship still remain as much of a frontier in science as ever. As we discover the interdependency of soil-biota relationships and nuanced effects on the performance of multiple functions, we start to realize the breadth of what we do not know about how our actions will impact ecosystems. In light of this, we suggest that future investigating the links between soil biodiversity and ecosystem functioning stability should more specifically pinpoint how the abundance of different populations of specific species of fungi and bacteria change across soil-biodiversity treatments and how the absence or presence of certain species help or hinder the temporal performance and stability of particular ecosystem functions.

Furthermore, monitoring how the asynchronous fluctuations of these specific soil species correspond with changes in the performance and stability of multiple ecosystem functions in model and natural systems would also be an obvious next step forward. A good methodology to achieve these goals would be to utilize the rapid technological advances that are being made in the world of molecular analysis. There now exist tools that can identify specific species of fungi and bacteria and allow scientist to track their abundance over time. As these methods become more financially obtainable, molecular ecologists around the world are starting to be able to work simultaneously on building a pool of knowledge that can be cross-compared to help us understand how the exact soil community compositional changes that occur over time can be methodically tied to fluctuations in the performance and stability of different ecosystem functions.

In line with previous studies, and based on our findings that our site-based multifunctionality analysis changed depending on the methodology and thresholds we used to calculate multifunctionality, we recommend that future studies employ a range of analysis techniques so that patterns and correlations are not overlooked (Hector & Bagchi 2007; Isbell *et al.* 2011; Hooper *et al.* 2012; Bradford *et al.* 2014; Allan *et al.* 2015). We suggest that all considered ecosystem functions be analyzed and presented individually to avoid the oversimplification and implicit equalizing of the value of all functions that happens in a multifunctionality calculation. Different ecosystem functions hold different value for different stakeholders (Lindemann-Matthies, Junge & Matthies 2010; Junge *et al.* 2015). For example, farmers might value biomass production, while recreational managers might value plant diversity more. Therefore, it is important not to generalize what is considered positive or negative functioning as well as construct arbitrary levels of what is considered successful functioning in a system. This is where the multiple threshold approach used in this thesis will prove to be useful in future studies. We also encourage continued research into the

mathematical reasoning and implications underlying these arithmetical methodologies so that the most reliable and informative technique is identified and used in the future to allow for studies to be cross-compared. This will also prove essential for making the field of ecology more translatable to people outside the realm of science so that the appropriate stakeholders and politicians can be engaged and better understand the implications of soil degradation without misinterpreting the predictions that scientists make (Mouquet *et al.* 2015).

Furthermore, as our knowledge of the particular links between guilds of soil microbial species and the stability of various ecosystem functions progresses, we encourage scientists to take this knowledge into the field to test concepts and theories in applied situations. Based on the increased number of variables that can influence the results of field experiments, we recommend many experiments be performed with high numbers of site replication to create a larger pool of data from which broader and more reliable conclusions can be drawn. This will be critical for investigating how differences in land-management practices truly affect soil community composition and how these differences are linked to changes in the stability of multiple ecosystem functions.

Considering the density of life that exists within soils and how much we rely upon the services they provide, the negative effects that soil degradation can have on global soil biodiversity levels as well as the provisioning of ecosystem services are exponential. However, looking at this grim fact from a positive angle, efforts that go into maintaining soil biodiversity can have great positive implications on maintaining the functioning of the natural world, which in turn can ensure the sustainability of services that we get from soil. By picking apart the intricate mechanisms that underlie how soil biodiversity is involved in provisioning the stability of ecosystem functions from which we benefit, soil ecologists can have tangible positive impacts on society and other life on this planet. Due to how much we rely on soil for our daily sustenance and existence, it is critical that we recognize soil

biodiversity as an invaluable resource that should be investigated and invested in so that it can be properly maintained to benefit future generations.

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Appendix

"The soil is the base of our existence. Everything we eat, drink, breathe and wear regularly comes into contact with the soil"

– Wim van der Putten

ART | 2012

The Effects of Agricultural Soil Communities on the Stability of Ecosystem Functioning

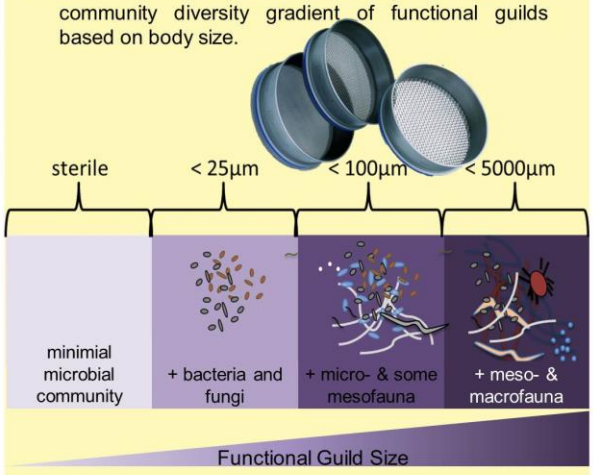
Sarah Pellkofer[1,2], Cameron Wagg[1,2], Bernhard Schmid [2], Marcel van der Heijden[1],
[1]Agroscope Reckenholz-Tänikon Research Station ART, CH-8046 Zürich; www.agroscope.ch
[2]University of Zürich, Institute of Evolutionary Biology and Environmental Studies, CH-8057 Zürich; www.ieu.uzh.ch

Project Questions

- How do differences in the diversity of soil communities under various agricultural management systems affect ecosystem functioning?
- How does ecosystem functioning change when the diversity of the soil community is manipulated to exclude specific soil functional guilds?
- How do these differences in 1.) the agricultural management systems and 2.) the soil community diversity affect the stability of ecosystem functioning when the system is stressed by changes in abiotic factors, such as periods of drought?

Methods

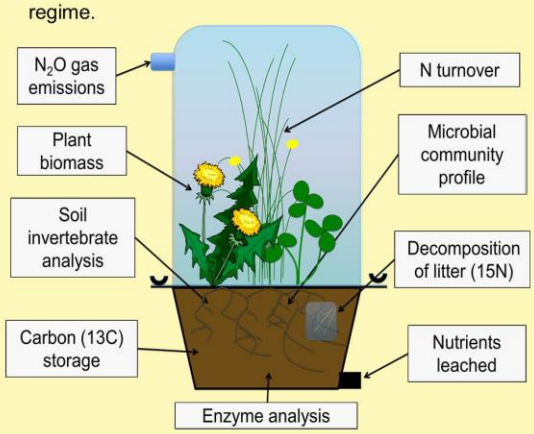
1. Soil was taken from 3 agricultural sites with different management practices: 1.) German Intensive, 2.) Swiss Conventional, 3.) Swiss Organic.
2. Each site soil was sieved to create a 4-tiered soil community diversity gradient of functional guilds based on body size.



3. Identical plant communities were grown in each soil treatment within EcoTubes to maintain soil biodiversity differences.

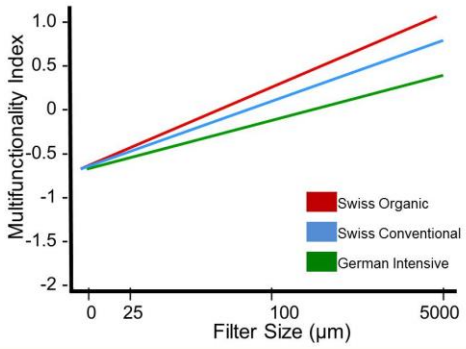
Methods (cont.)

4. A reduced watering regime was implemented later in the growing season.
5. Various ecosystem functions (quantified collectively as a single multifunctionality index) will be measured for each EcoTube before and after the reduced watering regime.



Expected Results

- Increasing soil biodiversity = increased ecosystem functioning and stability.
- Soil biodiversity will be greatest in Swiss Organic soils and lowest in German Intensive soils.



Conclusions

This experiment is still in progress and full results are expected in late 2013. The anticipated findings will demonstrate how agricultural management practices alter the functioning and stability of ecosystems through the differences they cause in soil biodiversity.

Questions or comments? Contact Sarah Pellkofer at sarah.pellkofer@ieu.uzh.ch

Agroscope

University of Zurich | Zurich – Basel Plant Science Center | FNSNF | Schweizerische Eidgenossenschaft Confédération suisse Confederazione Svizzera Confederaziun svizra Swiss Confederation | Federal Department of Economic Affairs FDEA Agroscope Reckenholz-Tänikon Research Station ART

Agroscope, Plant-Soil Interactions | 2014

Soil biota promote stability and species richness in an experimental grassland

Sarah Pellkofer[1,2], Cameron Wagg[1,2], Bernhard Schmid[2], Marcel van der Heijden[1,2]
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1. What We Already Know

Plant diversity positively affects net primary production (NPP) and stability of plant communities.
Soil communities positively affect the NPP, richness and evenness of plant communities.

2. What We Want to Know

Do soil communities also play a mechanistic role in **stabilizing** the NPP of plant communities?

3. How We Tested This

We grew identical plant communities in sterilized soil inoculated with
1) an unaltered, ("complex") soil community, or
2) soil sterilized ("simplified") to reduce community abundance & complexity.

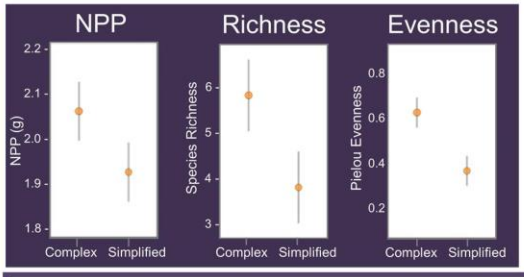
Plant NPP, richness, evenness, community stability and species asynchrony were assessed over 1-year.

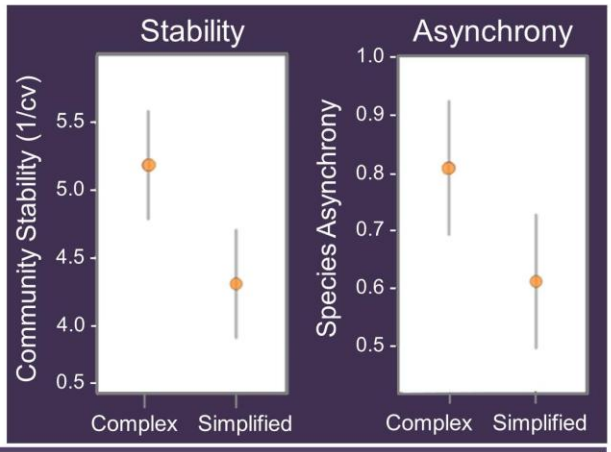
4. Hypotheses

The presence complex soil biota will support
1. higher NPP,
2. a higher diversity of plants that are more evenly represented, and
3. NPP that is more stable over time as a result of higher species asynchrony.

5. Results

The complex soil community enhanced plant NPP, richness, and evenness, as well as species asynchrony and overall NPP community stability.





6. Conclusions

Complex soil communities increase the overall productivity, diversity and stability of aboveground plant communities. Therefore, their preservation may be key for success of future management efforts to combat biodiversity loss and maintain the stability of terrestrial systems in the face of a changing climate.

Questions or comments? Contact Sarah Pellkofer at sarah.pellkofer@ieu.uzh.ch

Agroscope good food, healthy environment

University of Zurich | Zurich – Basel Plant Science Center | FNSNF | Schweizerische Eidgenossenschaft Confédération suisse Confederazione Svizzera Confederaziun svizra | Swiss Confederation | Federal Department of Economic Affairs, Education and Research EAER Agroscope

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“An understanding of the natural world and what's in it is a source of not only a great curiosity, but great fulfillment.”

– David Attenborough

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